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EDITED BY
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of the Agricultural Research Station, Rehovot, Palestine

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CONTENTS

STUDIES ON CERTAIN BUTYRIC ACID PRODUCING ORGANISMS ISOLATED FROM HEMP AND SIMILAR VEGETABLE FIBRES *)

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(With Plates II and III).

INTRODUCTION

Hemp and similar vegetable fibres appear to be a good source for sporulating anaerobic organisms producing acetone, butyl alcohol and butyric acid among other fermentation products from carbohydrate media. On such media as maize or potato mash or milk, these so-called butyric organisms present on the fibres develop to such an extent that they suppress other organisms and can therefore be isolated with comparative ease. This opinion was reached as the result of numerous isolations of butyric acid organisms under anaerobic conditions from hemp, jute and flax from various countries.

Many of these organisms were obtained in pure culture and closely observed. The cultures did not appear to be all identical. Some showed certain distinguishing features which have been retained after repeated subplantings in yeast glucose agar over a number of years.

There is a vast and confusing literature concerning the butyric organisms which complicates the recognition and classification of new strains and tends to bewilder the newcomer to this field of study. The scope of this paper is to describe in some detail some of our

*) Received for publication in November 1942.

cultures, compare them with the diagnoses given for similar if not identical organisms, and to discuss their taxonomy and nomenclature particularly with the view towards simplifying the recognition and naming of members of this large group of butyric acid forming organisms.

Before proceeding with the full description and discussion of these organisms, we review briefly the literature on similar organisms obtained from vegetable fibres. The study of clostridial forms on flax, hemp and other vegetable fibres was stimulated long ago by the interest aroused as to their actual role in the retting process of technical fibres, and diverse opinions regarding their retting abilities led to a sharp controversy as to their fermentability of pectin and cellulose.

FRIEBES (38) in 1895 was perhaps the first to isolate a plectridial butyric acid organism from retting flax, and from his researches he concluded it to be the specific agent of retting, but earlier workers, as TRECUL (30) in 1865 and VAN TIEGHEM (32) in 1877, observed amylobacter-like forms associated with the maceration and rotting of plant tissues and recognised their significance in the retting industry.

TRECUL grouped his so-called amylobacter-like organisms, all of which stained blue with iodine, into three sub-genera: *Urocephalum* contained the tadpole or drum-stick plectridial forms (probably the same as FRIEBES' organism); *Amylobacter* included the cylindrical forms and *Clostridium* the spindle forms. VAN TIEGHEM's *B. amylobacter* which embraced TRECUL's amylobacter forms was strongly criticised by OMELIANSKI (24) as a mixed species and not a cellulose fermenter as claimed by VAN TIEGHEM.

BEHRENS (2) reviewed the literature on the natural retting methods, and described a clostridium which he regarded as the retting agent of hemp. He invariably found clostridia, that is organisms of the amylobacter group (BEIJERINCK's *Granulobacter;* VAN TIEGHEM's *B.amylobacter;* TRECUL's *Clostridium*) associated with retting fibres. Once, however, he obtained a tadpole-like sporangial form similar to FRIEBES' organism.

STOERMER's (29) drum-stick-like *Plectridium pectinovorum*, perhaps identical with FRIEBES' organism, was isolated from retting flax and claimed to be the causal organism of the retting process. STOERMER found that his plectridium produced comparatively large quantities of volatile acids, as butyric and acetic, from glucose and galactose.

BEIJERINCK and VAN DELDEN (5) described a retting organism which they named *Granulobacter pectinovorum*. This organism is synonymous with *Cl. pectinovorum* Beijerinck and van Delden (Donker) and not to be confused with STOERMER's organism, since apart from certain morphological differences, the latter is a gelatine liquefier

whereas BEIJERINCK's retting organism was described by BREDEMANN (7) and DONKER (8) as a non-liquifier of gelatine.

CARBONE and TOMBOLATO with their reddish coloured *Cl. felsineum* isolated from retting hemp aroused further interest in these retting clostridia. Except for its reddish pigment (orange-yellow in alkaline media) this organism is closely similar to the acetone-butyl alcohol producing organism *Clostridium acetobutylicum* Weizmann.

RUSCHMANN and BAVENDAMM (26) isolated a number of so-called amylobacters from seeds, straw, vegetable and retting fibres of flax. They classified the organisms as *B.amylobacter liquefaciens* and *B.amylobacter non-liquefaciens* according to their ability to liquefy gelatine. On all media the former showed only plectridial forms, the latter only clostridial forms. The authors asserted that *Cl. amylobacter liquefaciens* was a causal agent of retting, and also confirmed the retting ability of CARBONE's *Cl. felsineum*. In all probability *Cl. amylobacter liquefaciens* is the same as STOERMER's *Pl. pectinovorum*. It is interesting to note that SJOLANDER and MCCOY (27) working with RUSCHMANN's strain of *Plectridium pectinovorum liquefaciens*, found that this organism, a supposedly pectin fermenter according to its name, was unable to utilize this carbohydrate in artificial culture.

METHODS OF ISOLATION AND PURIFICATION

To isolate these bacteria pieces of well-washed fibre material were put into tubes containing maize mash or milk, and anaerobically sealed according to DORNER and RITTER's method (9), using an absorbent cotton-wool plug, soaked with pyrogallol and soda solution, above the pushed-in non-absorbent plug of the tube. The tubes were tightly fitted with rubber corks and cultured at 37°C. Within 24 hours or so active gassing took place, which when strong forced out the corks. Those tubes were selected which showed the most vigorous evolution of gas.

For the further isolation and purification of these organisms, smear cultures were made on three successive yeast-glucose-agar slopes from the tip of a sterilised platinum needle dipped into one of the active cultures. The tubes were then anaerobically sealed and incubated at 37°C. Generally within 24 to 48 hours well developed colonies were formed which were fewer and more separated in the second and third tubes and consequently could be easily picked at and further subcultured in the same manner.

In this way the various organisms were isolated having these features in common that they were all anaerobic spore-formers and produced from glucose varying amounts of butyric and acetic acids, isopropyl, butyl and ethyl alcohols, acetone, and carbon dioxide and hydrogen gases.

For a clearer comparison of the cultures their diagnostic features are given under one descriptive chart.

DESCRIPTION OF THE ORGANISMS

Source: Culture A - Palestine jute
Culture B - Indian flax
Cultures C, D, E, F, G, H - Indian hemp
Cultures I, K - Manila hemp.

Morphology

Carbol fuchsin stained preparation from cultures grown on yeast-glucose-agar medium at 37°C were used.

Vegetative cells: Ends: rounded. Form and arrangement: see table I. Size: see table II.

TABLE I

Form and arrangement of vegetative cells

Culture	Form	Arrangement
A, H, B.	short and long, slightly curved	single, short chains
C, D, K.	short and long, slightly curved	single, chains
I.	short and long, slender and stout	" "
E, F.	mostly long and slender	" "
G.	short and long	single, chains showing tendency to adhere together in parallel formation.

TABLE II

Size of vegetative cells

Culture	Limits of size (microns)	Average (microns)
A	1.7 - 7.0 by 0.4 - 0.7	4.2 by 0.5
H	2.2 - 6.3 by 0.5 - 0.7	3.8 by 0.6
B	1.7 - 9.1 by 0.4 - 0.7	3.7 by 0.6
C	2.1 - 4.6 by 0.5 - 0.6	3.2 by 0.63
D	2.0 - 7.5 by 0.5 - 0.7	3.5 by 0.65
K	2.0 - 9.2 by 0.6 - 0.8	4.6 by 0.7
I	2.8 - 10.0 by 0.6 - 0.8	4.7 by 0.7
E	3.2 - 10.2 by 0.5 - 0.8	E, F. show tendency for long individual rods of about 0.6 microns width
F	2.0 - 16.0 by 0.5 - 0.8	
G	2.0 - 6.0 by 0.5 - 0.7	3.2 by 0.5

Sporangia: Present. Cultures E and F showed poor sporangial production. Form and size: see table III and plate III, figs. 4-7.

TABLE III *Form and size of sporangia*

Culture	Form	Limits of size (microns)	Average size (microns)
A	plectridial	5.8-10.0 width of tail	6.9
H	plectridial	6.0-10.0 varies from 0.7 microns up to	7.7
B	plectridial	5.6-14.2 spore width	7.8
C	mainly spindle-shaped, also elliptical	4.1- 8.3 by 1.0-1.5	5.2 by 1.27
D	ditto	3.6-5.8 by 0.9-1.8	4.75 by 1.2
K	varied, long and slender, short and stouf	4.3-9.7 by 0.9-1.3	6.5 by 1.1
I	varied, lemon shaped, elliptical, greatest width at centre, relatively few swollen towards one end, highly granulated	4.0-9.3 by 1.0-2.1	5.6 by 1.4
E	elliptical, certain amount club-shaped or irregular	4.8-8.5 by 0.9-1.3	6.2 by 1.1
F	ditto	3.8-11.4 by 1.0-1.4	5.6 by 1.3
G	mainly elliptical, also spindle shaped	3.7-7.6 by 0.7-1.6	5.6 by 1.0

Endospores: Present. Location and size: see table IV.

TABLE IV *Location and size of endospores*

Culture	Location	Limits of size (microns)	Average size (microns)
A	terminal	1.4 - 2.7 by 1.0 - 1.8	2.1 by 1.43
H	terminal	1.7 - 2.8 by 1.1 - 1.6	2.3 by 1.4
B	terminal	1.2 - 2.3 by 1.0 - 1.5	1.8 by 1.2
C	subterminal: relatively few central; several instances observed of young spores rotating on their long axis within the sporangium;	2.2 - 3.1 by 1.0 - 1.3	2.4 by 1.2
D	ditto	2.0 - 3.0 by 1.0 - 1.4	" "
K	ditto	2.0 - 2.7 by 1.0 - 1.4	" "
I	mostly central to sub-terminal; often exhibits rotary movement on long axis within the cell	2.0 - 2.9 by 1.2 - 1.5	2.4 by 1.3
E	mostly sub-terminal; vegetative "tails" persist for considerable time	2.0 - 2.9 by 1.0 - 1.6	2.6 by 1.2
F	ditto	2.3 - 3.0 by 1.2 - 1.4	2.7 by 1.3
G	mostly central also subterminal.	1.6 - 3.0 by 1.0 - 2.1	2.2 by 1.2

Motility: Motile.
Flagella: Present in all cultures, peritrichous. Stain: Cesares-Gil.
Irregular forms: Present in B, C, D, K, E and F. (see plate III, fig. 8).
Staining reactions: All Gram positive in young cultures. Lugol's iodine: young vegetative cells stain yellow, granulose in clostridia stains blue-violet.

It is evident that according to their morphology the cultures tend to show a certain natural grouping. Cultures A, B and H because of their plectridial form, resembling drum-sticks or tennis rackets, with the spore borne at the swollen tip, form together a clearly defined group. The morphological grouping of the remaining cultures is not marked. Culture G may perhaps be separated from the others by producing generally more or less regular, elliptical sporangia with central spores; the rods appear to be more or less uniform and stained preparations tend to show the rods adhering together in parallel formation. Culture I has also elliptical sporangia but these are stouter and characteristic. When young the protoplasm of the whole cell is densely granulated, but in later stages of development the granulated protoplasm becomes concentrated in one protoplasmic mass to form the spore and may often be observed rotating fast on its long axis within the sporangium. The mature spore, however, has a thick wall and occupies the full width of the sporangium. Although these characteristics are not exclusive to culture I, yet they appear to be more general in this culture than among the others.

The tendency for long individual rods and the production of relatively very few sporangia, the vegetative part of which persists for a considerable time giving a tail effect to the spore, bring together cultures E and F, and place them somewhat apart from cultures C, D, K, I and G.

Colonial form: Growth is visible within 24 hours on yeast-glucose-agar slopes under anaerobic conditions. After 48 hours the following natural grouping according to well defined differentiations in colonial form was observed:

(a) Cultures A and H: Large, rounded or irregular, raised, smooth or crested, often star-shaped, compact, glutinous, creamy-white, from several mm. to about 5 mm. diameter. Whole colony removed intact when fished with needle (pl. II, fig. 1c).

(b) Culture B: Small, circular, translucent, smooth, entire margin, about 1 mm. diameter.

(c) Cultures C, D, K, and I: Circular, more or less opaque, smooth, shiny, raised, creamy coloured, entire margin, slimy, later confluent and butyrous (pl. II, fig. 1b). Culture I tended to be less opaque than the others.

(d) Cultures E and F: Irregular, more or less undulate margin, translucent, granulated, several mm. diameter (pl. II, fig. 2).

(e) Culture G: Circular, opaque, smooth, shiny somewhat raised, yellowish-white, entire margin. Whole colony removed when fished with inoculating needle (pl. II, fig. 1a).

With perhaps the exception of culture I, the above grouping lends further support to the tentative grouping suggested by the differences observed in the size and form of the sporangia.

Cultural and physiological characteristics

Temperature range of fermentation: 20°—45°C. Optimum: 37°C.

Maize mash: A brief comparison of the behaviour of the cultures in maize mash is as follows:

Cultures A, H, B, C, D, K: partial diastatic action, rapid fermentation and active gas production. So called "head" formed above a somewhat turbid serum. Head collapses and cultures shows three layers: top clear serum, fine colloidal layer, bottom coarse-sediment (see plate II, fig. 3a). Odour: butyric.

Cultures E and F: Partial diastatic action, active gas production. Odour: butyric.

Culture I: Good but not complete diastatic action, head formed above fairly clear serum and slowly collapses. Odour: butyric.

Culture G: Rapid and complete diastatic action (no residual starch), very active gas production. Small head formed with slimy strands extending down into the clear serum (pl. II, fig. 3b). Yellowish pigmentation produced. Odour: butylic.

The behaviour of culture G on maize is one of its main diagnostic features, easily segregating it form the other cultures.

Potato mash: Similar to maize mash.

Milk: All the cultures coagulated milk, giving an acid reaction. The coagulum was broken up through active gassing and a certain amount of peptonisation took place. Cultures A, H, B, I and G gave a stormier fermentation than the rest.

Gelatine: The organisms all grew in glucose-gelatine medium.

Cultures A, H, B and G brought about a positive liquefaction of the gelatine within several days. Cultures C, D, E, F, I and K consistently failed to liquefy gelatine even after 21 days.

The cultures were grown in a medium containing 2% gelatine and 0.5% yeast-extract. Cultures A, H, B and G when tested with

trichloroacetic acid (25%) showed a slight turbidity after 24 hours and no turbidity after 48 hours as compared with a dense turbidity produced by the test reagent with cultures C, D, E, F, I and K and the control medium thus showing the complete hydrolysis of the gelatine by the former cultures and confirming the above gelatine liquefaction results.

Nitrate reduction: All the cultures were unable to reduce nitrates when grown on nitrate-peptone-broth. The test for nitrite by the addition of dimethyl-alpha-naphtylamine sulphanilic acid proved to be negative throughout.

Hydrogen sulphide production: The production of hydrogen sulphide from yeast-glucose-agar and from maize mash was tested by hanging moist lead acetate paper in the culture tube. Unimportant differences between strains were detected. Cultures A, H, B, I, E, F, G showed a slight blackening of the lead acetate paper indicating slight hydrogen sulphide production; cultures C, D, K showed traces of hydrogen sulphide. On yeast-glucose-lead acetate-agar no hydrogen sulphide could be detected.

Catalase: Cultures showing good growth on yeast-glucose-agar slopes under anaerobic conditions were tested for catalase production by the addition of hydrogen peroxide (3%). In no case was the hydrogen peroxide decomposed, thereby showing the absence of catalase.

Carbon metabolism: The ability of the cultures to ferment various carbohydrates and related substances in standard broth at 37°C was observed by noting the gas and acid production after 10 days. Our general observations are given in Table V.

TABLE V

Fermentation of carbohydrates and related substances

Substrate	A	H	B	C	D	K	I	E	F	G
Glucose	G	V.G	G	V.G	V.G	V.G	V.G	V.G	V.G	V.G
Laevulose	G	V.G	M	M	G	M	V.G	V.G	G	G
Galactose	G	V.G	M	F	M-G	S-M	V.G	F	G	V.G
Saccharose	V.G	V.G	M	G	G	M	G	G	G	G
Lactose	G	V.G	M	G	G	M	F	G	G	V.G
Maltose	G	G	M	M	G	M	V.G	G	G	V.G
Mannose	V.G	V.G	G	M	M	M	V.G	G	G	G
Raffinose	G	V.G	M	F	S	M	V.G	O	G	M
Arabinose	G	V.G	M	M	G	M-G	G	G	M	V.G
Xylose	S	V.G	M	M	G	G	N	G	M	V.G
Rhamnose	S	V.G	S	F	O	M	N	S	F	S
Starch	G	V.G	G	M	G	G	V.G	G	F-M	V.G
Inulin	S	S	S	F	S	F	M	F	G	F
Dextrin	G	V.G	M	F-M	G	G	O	G	G	V.G
Glycerol	S	S	S	O	O-S	S	M	F	F	S
Pectin	S	S	F	O	—	—	S	—	—	O

V. G = very good, G = good, M = moderate, F = fair, S = slight, N = negligible, O = negative.

Table V demonstrates that the majority of carbohydrates as saccharose, glucose, laevulose, galactose, lactose, maltose, mannose, arabinose, starch etc. are readily fermented by these organisms, whereas glycerol, pectin, inulin, and to a certain extent rhamnose are in general only slightly attacked, if at all.

Summarising our observations on the morphological, cultural and physiological characteristics of these organisms it can be seen that they fall naturally into three main groups.

Group I: Cultures A, B, H - plectridial butyric organisms which are actively proteolytic and are gelatine liquefiers. Strain B, however, shows a certain difference in its small colonial form on nutrient agar and may be regarded as forming a sub-group.

Group II: Cultures C, D, E, F, I, and K - clostridial butyric organisms which are moderately proteolytic on milk, have a more or less partial diastatic action on maize starch and are non-liquefiers of gelatine. Culture I, may perhaps be regarded as a sub-group since it appears to show certain differences in the clostridial form, and to be more active than the others on maize and glycerol, while cultures E and F have a characteristic colonial form and tend to produce long undivided threads on nutrient agar, and therefore may be considered as another sub-group.

Group III: Culture G - clostridial butylic organism, rapid and complete diastatic action on maize producing strong butyl odour, liquefies gelatine, actively proteolytic on milk.

FERMENTATION OF CARBOHYDRATES

A quantitative comparison of the fermentation products from various carbohydrates by these organisms was carried through in order to determine whether these organisms could be further identified by their fermentation products.

Experimental series 1. *Analyses of the products of fermentation of glucose.*

The medium used consisted of 1.0 gm. dried yeast autolysate 0.375 gm. asparagine, 20 gm. glucose in tap-water per litre. One litre medium was poured into a 1.5 litre flask, 2 gm. asbestos added and about 70 ml. removed into a 100 cc. flask, and sterilised at 110° for one hour. The small flask was inoculated with an active 24 hours old maize-tube culture, prepared from single colonies of the organism grown anaerobically on yeast-glucose-agar slopes, and within 24 hours further inoculated into the big flask and kept at 37°C.

Analytical methods:

Titratable acidity was determined by titrating 10 ml. of fermented medium with 0.1 N caustic soda using phenol-phthalein as indicator.

Unfermented sugar: Residual sugar was determined by STILES, PETERSON and FRED's (28) modification of the micro-method of SHAFFER and HARTMANN.

Solvents: 500 ml. fermented medium were neutralised and distilled and 200 ml. distillate collected. Acetone was estimated according to GOODWIN's (13) modification of MESSINGER's method; isopropyl alcohol by the method recommended by LANGLYKKE et al. (18) and ethyl and butyl alcohols by JOHNSON's (16) method.

Volatile acids: The residue after distilling off the solvents was acidified with sulphuric acid and steam distilled. 500 cc. distillate was collected and determined for solvents according to VIRTANEN and PULKKI's (34) modification of the DUCLAUX method. Formic acid was estimated directly from the fermented medium by the FINCKE method (23).

Lactic acid: FRIEDEMANN and GRAESER's (11) method was used for the determination of lactic acid.

Acetyl methyl carbinol: This was determined on 200 ml. of the fermented medium according to KLUYVER, DONKER and VISSERT HOOFT's (17) modification of LEMOIGNE's method.

TABLE VI

Fermentation products in 1 litre yeast asparagine glucose medium after 6-7 days incubation at 37°C.

	A	H	B	C	D	K	I	E	F	G
ml. of 0.1 N nitratable acid in 10 ml.	1.60	1.84	1.19	0.59	2.41	1.08	1.46	2.59	2.73	1.84
Glucose fermented%	16.25	20.45	11.20	75.90	27.60	76.40	100.00	25.80	28.15	100.00
	Calculated as percentage of glucose fermented									
Acetone	0.46	0.88	2.12	4.31	0.82	6.48	1.04	0.19	0.57	7.72
Isopropyl alcohol	1.72	2.20	3.26	2.52	2.74	2.12	10.00	1.29	3.97	1.23
Butyl alcohol	15.01	3.17	0.78	24.77	3.32	20.68	22.95	1.22	9.38	21.62
Ethyl alcohol	1.32	14.30	23.57	21.86	13.41	16.40	41.18	21.61	24.66	26.81
Butyric acid	17.76	30.64	17.07	1.74	22.08	2.82	2.60	7.42	20.80	2.79
Acetic acid	7.51	6.33	28.54	2.61	10.73	2.58	2.54	21.34	11.14	4.88
Formic acid	0.00	0.00	0.00	1.04	2.56	1.04	0.65	0.03	0.00	0.00
Lactic acid	5.21	0.35	10.65	1.98	0.00	1.22	0.23	1.58	0.66	1.12
Acetyl methyl carbinol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.07

The analyses given in Table VI show that all the organisms produced varying amounts of neutral products and volatile acids. All the cultures produced isopropyl alcohol. Organisms C, K, G and I appear to be good alcohol producers, the first three cultures also produced acetone whereas culture I produced considerably more isopropyl alcohol and far less acetone. These neutral solvents appear to be formed at the expense of acid production. Cultures E and F

produced slight amounts of acetone and isopropyl and butyl alcohols and better yields of volatile acids. Culture D and also cultures A, H and B also appear to be good volatile acid producers, and, except for culture A which gave good yields of butyl alcohol, these cultures appear to be poor producers of acetone and butyl alcohol.

Experimental Series 2. Analyses of the products of fermentation of glucose in the presence of calcium carbonate

Culture D was unfortunately lost. The medium per litre flask used was 600 ml. 2% glucose, 0.5% Difco's yeast extract and about 5 gm. calcium carbonate. The chalk was separately sterilised. 1 ml. of an active yeast glucose culture about 20 hours old was used as inoculant.

The end products of fermentation were analysed according to the methods given above, except for the estimation of volatile acids which was determined as follows: 50 ml. of the medium were acidified with phosphoric acid distilled until about 12 ml. remained. 50 ml. of CO_2 - free distilled water were added to the distilling flask and distillation continued. This was repeated until all volatile acids were distilled over. The volatile acids thus collected were titrated against 0.1 N NaOH. The acids were then set free by adding an equivalent amount of 0.1 N H_2SO_4 and the solution diluted so that 110 ml. contained about 10 ml. 0.1 N acid. The volatile acids were then determined according to DUCLAUX (10) and GILLESPIE and WALTERS (12). The distillation constants were calculated from our distillation figures obtained with pure acids.

TABLE VII

Fermentation products from 2% glucose in the presence of 0.5% *Difco's yeast extract and* $CaCO_3$ *after* 5-6 *days at* 37°*C.*

	A	H	B	C	K	I	E	F	G
Glucose fermented %	100	98.35	74.65	99.58	99.29	99.29	99.60	99.39	100
	Calculated as percentage of glucose fermented.								
Butyric acid	11.70	19.35	4.42	18.00	18.38	23.01	32.22	30.30	30.57
Acetic acid	14.41	8.78	7.54	11.16	8.87	9.80	10.47	7.38	13.91
Lactic acid	3.46	8.63	53.19	0.00	0.00	0.77	0.00	0.00	0.00
Formic acid	0.60	0.05	2.17	0.26	0.71	1.05	1.73	0.36	0.10
Acetone	4.47	1.38	1.02	1.12	0.94	0.94	0.05	0.05	0.05
Isopropyl alcohol	0.70	0.36	1.97	1.63	2.75	1.71	1.02	0.92	1.72
Butyl alcohol	7.43	9.44	2.24	10.79	11.78	9.16	1.17	2.85	0.83
Ethyl alcohol	3.77	5.46	12.09	5.37	6.00	4.79	5.44	4.02	7.45

The main point of interest from the results given in table VII is the relationship between butyl alcohol and butyric acid production by the clostridial forms. A higher butyric acid production is clearly

associated with lower butyl alcohol yields. The two products together accounted for about 29 to 33.5% of fermented sugar. Thus cultures E and F, which appear to be identical, and culture G produced about 30 to 32% butyric acid and approximately 1 to 3% butyl alcohol, whereas cultures C, K and I produced 18 to 23% butyric acid and approximately 9 to 12% butyl alcohol.

Another interesting observation is that the plectridial form B produced less volatile acids, and considerably more lactic acid than the other two plectridial cultures A and H.

The markedly higher production of isopropyl alcohol by culture I from glucose medium without calcium carbonate (see table VI) was not maintained on the addition of calcium carbonate to the medium.

Experimental series 3. Fermentation of maize-meal mash

Further experiments were conducted to test the ability of the cultures to produce neutral solvents from a starch substrate as maize-meal mash (table VIII).

TABLE VIII

The fermentation of 500 ml. 5% maize mash

Culture	N/10 acid of 10 ml. fermented medium	Yield of solvents	Solvent yield on available carbohydrate*)
	ml.	ml.	%
A	2.5	0	
	1.9	0	
H	3.0	0	
	2.7	0	
B	1.5	0	
	2.2	0	
C	1.6	0	
	1.5	0	
K	1.5	0	
	1.9	0	
D	0.8	0	
	1.0	0	
I	2.6	0	
	2.6	0	
E	1.4	0	
	1.7	0	
F	1.2	0	
	1.5	0	
G	2.8	6.4	34.1
	3.6	5.9	31.5

*) available carbohydrate - 15.0 gms.

The solvent yield was obtained by distilling over about one third of the total neutralised fermentation medium, adding common salt to the distillate and re-distilling. The volatile solvents were then salted out from the second distillate by saturating with potassium carbonate thereby causing the solvents to separate out as an oily layer which could then be measured.

Only culture G produced neutral solvents, although all cultures produced volatile acids.

Experimental series 4. Fermentation of sugar mashes

A comparison was made of the abilities of the various cultures to ferment certain sugar mashes, as cane sugar in molasses and lactose in whey (tables IX and X). Untreated molasses gave negative or negligible yields of neutral solvents even in the presence of such so-called activator substances as soya-meal, whereas hydrolysed molasses proved to be a suitable fermentation medium for the production of neutral solvents by some of the organisms. Hydrolysis was carried out by treating the molasses mash with 0.5% conc. HCl, then steam-heating, for one hour, after which the mash was neutralised with ammonia. The whey medium was neutralised with ammonia.

TABLE IX

The fermentation of 500 ml. 5% inverted Belgian beet molasses (sugar content - 48.5%) with the addition of 0.5% soya meal and kept at 37°C.

Culture	N/10 acidity of 10 ml. fermented medium	Yield of solvents	Solvent yield on available sugar
	ml.	ml.	%
A	1.6	0	0
	1.6	0	0
H	2.7	0	0
	2.9	0	0
B	3.1	0	0
C	1.1	5.45	36.0
	1.3	5.70	37.7
	1.0	5.30	35.0
	1.0	5.5	36.4
D	3.2	1.8	11.9
	3.2	2.0	13.2
K	2.2	5.05	33.3
	2.1	5.0	33.0
I	4.8	2.3	15.2
	4.9	2.1	13.9
	4.2	3.5	23.1
E	3.6	traces	-
	3.6	traces	-
F	3.9	0.4	-
	3.6	0.1	-
G	5.0	0.3	-
	4.9*	0.6	-
	5.4*	traces	-

*) Inverted Belgian cane sugar molasses used.

TABLE X

The fermentation of 500 ml. whey with the addition of 0.5% soya meal.

Culture	N/10 acidity of 10 ml. fermented medium	Yield of solvents	Solvent yield on available sugar
	ml.	ml.	%
A	5.0	0.5	2.4
H	5.4	1.6	7.7
	2.6	0.4	1.9
B	2.8	0.0	0
	2.2	traces	-
C	3.2	3.1	14.6
D	4.0	0.4	1.9
	2.2	3.0	14.1
K	4.2	1.0	4.8
I	2.0	0.3	1.4
E	6.4	2.2	10.6
F	1.8	0.3	1.4
	3.85	0.4	1.9
G	3.8	7.5	35.3
	3.8	7.4	34.8
	3.3	7.9	37.2
	2.8	8.4	39.5

The results show that the cultures differed in their abilities to attack inverted molasses and lactose in whey. Organisms C and K can be considered good solvent producers from inverted molasses. Cultures I and D are also capable of fermenting inverted molasses. Organism G proved to be the best of the series for the production of solvents from whey.

GENERAL DISCUSSION

Our data give sufficient proof that the described organisms fall into three natural closely related groups having many features in common. They are gram positive, granulose-containing anaerobic spore-formers, which are able to ferment carbohydrates with the production of carbon-dioxide and hydrogen gases, varying amounts of neutral solvents, as acetone, butyl, isopropyl and ethyl alcohols, and also volatile organic-acids, as butyric and acetic. Other end products such as formic and lactic acids may or may not be formed by the organisms. Their major differences, however, clearly demarcate them into distinct groups. Some of the cultures can be recognised by certain minor features which we believe do not justify their complete separation from the main groups, but could be considered as sub-groups.

Group I. Cultures A, H, B. These strains obviously fall into one natural group. They can be easily distinguished by their plectridial form resembling drum-sticks or tennis rackets. The crested, often

star-shaped, glutinous, semi-translucent colonies of strains A and H on yeast-glucose-agar under anaerobic conditions are unusual among butyric acid bacteria (pl. II, fig. 1c; pl. III, fig. 4). Under the same conditions the colonial form of strain B is, however, noticeably different; this strain invariably produced small rounded translucent colonies.

As a group they are gelatine liquefiers. They are poor to fair fermenters of carbohydrates in the absence of chalk, and may produce certain amounts of neutral solvents from sugar media. The higher production of lactic acid from glucose by strain B again marks off this strain from A and H. Consequently strain B may be considered as a sub-group.

A detailed account of these plectridia including a discussion on their taxonomy has already been described (WEIZMANN and HELLINGER (36)).

Group II. Cultures, C, D, E, F, I, K. One of the outstanding characteristics of this group is that they do not liquefy gelatine. This group is further subdivided for reasons given below.

(a) *Cultures C, K and D.* Strains C and D produce typical spindle or club shaped clostridia with terminal or subterminal, rarely central spores (pl. III, fig. 5). Strain K, however, deviates from the typical form by showing somewhat varied stout and slender elliptical forms and a certain number of evolution forms. Yet C and K agree sufficiently in important morphological, cultural and physiological features to be grouped together.

Among the fermentation products of glucose by strains C and K, the yields of butyl alcohol were highest, closely followed by ethyl alcohol. Acetone was produced in smaller amounts, and isoproply alcohol in least amounts. These two strains proved to be the best fermenters of inverted molasses.

Strain D which was only a fair fermenter of glucose and sugar mashes, formed from the fermented glucose comparatively higher yields of ethyl than butyl alcohol. Acetone yields were very slight. Butyric acid was formed in proportionately higher amounts than the other fermentation products. The strain may perhaps be regarded as a weak member of this group.

(b) *Culture I.* Although similar to subgroup (a) in its salient characteristics, culture I can on several counts be distinguished as a distinct strain from C, K and D. Firstly, strain I could always be recognised by its stout, somewhat lemon-shaped clostridia exhibiting a highly granular protoplasm which in the mature sporangium becomes concentrated towards one half of the cell and in the remaining part the developing spore lies within a clear protoplasmic mass (pl. III, fig. 6). As previously noted this characteristic was not exclusive to this strain, but was more general here than among the other strains.

Secondly, strain I fermented glycerol. Thirdly, from glucose medium without chalk, the strain produced considerably more isopropyl alcohol than all the other strains and also higher yields of ethyl alcohol.

(c) *Cultures E and F.* These cultures can be distinguished by their semi-translucent granulated uneven colonies, and the tendency to produce very long rods accompanied by scarcely any sporulation on yeast-glucose medium (pl. II, fig. 2; pl. III, fig. 7). The organisms are fair fermenters of glycerol and produce relatively large amounts of ethyl alcohol and butyric acid from glucose. The two strains, however, appeared to differ in their fermentability of whey and showed slight differences in isopropyl and butyl alcohol yields from glucose.

Group III. Culture G. This group is easily recognisable by its active fermentation ability on maize mash, its rapid and complete diastatic activity and its characteristic behaviour in maize producing a strong butyl smell. In fact this group only can be considered a producer of acetone and butyl alcohol from maize starches. This group can be further identified by its ability to liquify gelatine within 24 to 48 hours. A further differential test is its growth on nutrient agar surface, — the creamy compact colonies are removed whole when fished with a needle. The microscopic features, too, are characteristic. The sporangia are generally slender and elliptical and the spores are mainly central (pl. III, fig. 9).

Culture G proved to be a poor fermenter of molasses, but produced satisfactory yields of acetone and butyl alcohol from whey.

NOMENCLATURE AND TAXONOMY

Since all the strains are anaerobes and the rods are swollen at sporulation producing clostridium or plectridium forms, these strains undoubtedly belong to the generic group of *Clostridium* (BERGEY (6)). Comparing the organisms with previously described forms we classify our cultures as belonging to the following specific groups, a full discussion of which is given below.

I Cultures A & H	*Cl. pectinovorum* Stœrmer
Culture B	variety of *Cl. pectinovorum*
II Cultures C, D, K	*Cl. butyricum* Prazmowski
Culture I	variety of *Cl. butyricum*
Cultures E & F	variety of *Cl. butyricum*
III Culture G	*Cl. acetobutylicum* (Weizmann) McCoy

Clostridium butyricum. This is a large and variable group of organisms which are known as the true butyric organisms. In general they produce slight yields, if any, of neutral volatile products from starch media, and show great variation in their ability to ferment sugar media. Many attempts have been made to classify this group. BREDEMANN's (7) exhaustive study on *B.amylobacter* (1909) was the first serious attempt to group together some of these

identical but variously named organisms. He studied many original strains of other investigators including *Cl. amylobacter* I. Grueber; *B. saccharobutyricus* v. Klecki; *Granulobacter pectinovorum* Beijerinck et van Delden etc. From their morphological and physiological details BREDEMANN came to the conclusion that although minor differences could be observed between different strains, differences which were not constant in any one strain and could be observed among other strains, there was no clearly defined difference to warrant their consideration as separate species. To exemplify this: WINOGRADSKY (39) distinguished his strain *Cl. pastorianum* from other butyric acid organisms on the basis that his strain showed a peculiar persistant triangular capsule attached to the spore. But PRAZMOWSKI had observed this feature among spore stages of *Cl. butyricum*. GRUEBER refers to a membrane in his *Cl. amylobacter* and BREDEMANN also observed that the presence of a capsule varies among individuals in the same species even under the same conditions.

BERGEY in his Manual of Determinative Bacteriology (6) added many more butyric acid organisms to those identical with *Cl. amylobacter* A. & M. Bredemann, and cited them all as synonyms of *Cl. butyricum* Prazmowski. In the 1934 edition of the Manual, BERGEY claimed that *Cl. butyricum* is able to reduce nitrates, that the spores are central and the rods not swollen at sporulation. Our observations did not substantiate these claims. BREDEMANN in his monograph, and MCCOY and collaborators (19, 21), also record the inability of these organisms to reduce nitrates. Most investigators agree that these true butyrics have spindle or club-shaped or elliptical sporangia with excentric to subterminal spores. In the latest edition of BERGEY's Manual (1939), these statements have been corrected so that BERGEY's diagnostic features for the group are in line with considered opinions.

The taxonomy of the butyric acid organisms has been closely studied by American investigators, especially MCCOY and co-workers. MCCOY, FRED, PETERSON and HASTINGS (21) distinguished between several physiologic types of the so-called true butyric anaerobes as follows: (1) *Cl. pasteurianum* type which does not ferment starch, (2) *B. saccharobutyricus* type which partially hydrolyses starch and (3) a group of starch fermenting plectridia which the authors leave unnamed, but which we believe are in all probability identical with the species *Cl. pectinovorum* (WEIZMANN & HELLINGER (36)). Contrary to the claim that the *Cl. pasteurianum* type does not ferment starch, SJOLANDER and MCCOY (27) studying three strains in their possession which had been classified as *Cl. pasteurianum* found that all three gave the typical *B. saccharobutyricus* type of fermentation. These strains also fermented lactose, whereas MCCOY and co-workers (20,21) considered the *pasteurianum* type as unable to ferment lactose in agreement with WINOGRADSKY's (39) diagnosis of the original strain of *Cl. pastorianum*. The 1934 edition of BERGEY's Manual gave *Cl.*

pasteurianum as a synonym of *Cl. butyricum* Prazmowski and therefore a lactose fermenter, but the latest edition considers *Cl. pasteurianum* as a sub-group possessing the general characteristics of *Cl. butyricum* and the distinctive characters of prolonged retention of the spore within a peculiar brush-like spore capsule, and the non-fermentation of starch, lactose, glycerol and mannitol. Apropos the non-fermentability of starch by certain butyric strains, mention may be made of the diverse views regarding the starch fermenting ability of another true butyric called *Cl. beijerinckii*. KLUYVER's strain of this organism was originally described as a non-starch fermenting butyric (8). VAN DER LEK (31), from the same laboratory, reported the hydrolysation of starch by this organism. SJOLANDER and MCCOY's (27) experiences with two cultures of *Cl. beijerinckii* from the Delft laboratory agreed with DONKER's description that the organism is unable to ferment starch. Our own observations on a strain of *Cl. beijerinckii* Donker from the Lister Institute's National Collection of Type Cultures were that the organism when first received in 1934 was able to attack starch, but after a number of years of subplanting on yeast-glucose-agar the organism appears to have lost its ability to ferment starch. BERGEY has revised his former decision of regarding *Cl. beijerinckii* as a synonym of *butyricum* and places it in a separate subgroup of *butyricum* with the distinctive character of being a non-fermenter of starch and glycerol differing from *pasteurianum* in being able to ferment lactose.

MCCOY and co-workers' (21) classification of the true butyrics does not find support in their and LANGLYKKE's grouping of the butyrics according to their products of fermentation of glucose and arabinose. Members of both the *pasteurianum* and *saccharo-butyricum* types are distributed among the sub-sections of the two groups (1) the isopropyl alcohol producers with or without acetone production and (2) the acetone producers. Both groups however include also the production of butyl and ethyl alcohols. Some of the members are poor fermenters, others are fair to good. When we consider the variations in the yields of neutral solvents by these butyrics and also the marked discrepancies in the metabolic products obtained by various investigators working independently with the same organisms, we naturally hesitate in placing undue emphasis on this procedure as a means of diagnosing species of the butyrics. Early investigators drew attention to these variations. BEIJERINCK (3) in 1893 at first claimed that his *Granulobacter butylicum* produced pure butyl alcohol accompanied by smaller amounts of lower boiling alcohols. In a later paper BEIJERINCK (4) mentioned that these organisms produced larger quantities of n-propyl alcohol than butyl alcohol. WINOGRADSKY (39) also showed how different the ratios of the obtained alcohols can be under not so different conditions.

Referring to our butyric organisms, they all produce acetone, isopropyl, butyl and ethyl alcohols from glucose. Cultures

C and K produced butyl alcohol in greatest amounts closely followed by ethyl acohol, much smaller amounts of acetone, and least amounts of isopropyl acohol. Cultures D, I, E, F gave higher yields of ethyl alcohol than butyl alcohol and least yields of acetone. Culture I proved to be the best fermenter among our organisms, producing good yields of ethyl alcohol, smaller but also good yields of butyl alcohol and a considerable amount of isopropyl alcohol as compared with acetone. Since they all ferment starch, they should, according to BERGEY, be designated *Cl. butyricum*. Two of the strains, namely E and F, can be distinguished from the others by certain minor but characteristic features. These strains also showed a marked scarcity of spore production. Instances occurred during sub-culturing of increased sporulation, but this could not be maintained. Direct plantings from sporulating cultures still gave the typical colonial form on nutrient agar slopes. WINOGRADSKY (39) noted a tendency for degeneration in cultures of his particular organism resulting in rods developing into threads, and a retardation of clostridia and spore formation. According to him once spore formation disappears, it cannot be restored. The organism has, so to speak, developed an asporogenous form in which one could hardly recognise the earlier characteristics, in fact it changes physiologically as well as morphologically. From our own experience sporulation is not, however, altogether lost.

These observations and views tend to caution us in separating the strains E and F from the others, yet in view of the fact that the two strains can be quickly recognised by their colonial form, we feel justified in regarding them as a variety of *Cl. butyricum*, and suggest the varietal name of *elongatum* thereby drawing attention to the elongated rods and threads which characterise them on nutrient agar media.

Culture I which shows a good diastatic action on maize, and has after about eight years still retained the ability to ferment to a certain extent glycerol, and produces noticeably more isopropyl alcohol from glucose than our other cultures, could also be distinguished by its characteristic sporangial form. There is a very close resemblance between the clostridia of culture I and the drawings and descriptions of the swollen short or long elliptical, highly granulated sporangia with central to sub-terminal spores of WINOGRADSKY's (39) *Cl. pastorianum*.

It may be noted that WINOGRADSKY's drawings and description do not agree with the sporangial form of a number of cultures labelled *Cl. pasteurianum* (or *pastorianum*) from various well-known collections. These cultures exhibit more the spindle to club-shaped sporangia bearing excentric to sub-terminal spores typical of *Cl. butyricum* including our strains C, D and K (pl. III. figs. 4, 6). However, culture I could not be classified as variety *pasteurianum* since it

ferments starch and glycerol, which *pasteurianum* is characterised as being unable to do. As true strains of *Cl. butyricum* are considered to be non-fermenters of glycerol we suggest for culture I the name *Cl. butyricum* variety *glycerolyticum* because of its ability to ferment glycerol.

In reviewing the patent literature, the descriptions given of such technical species as *Cl. saccharobutylicum beta* (Izsak (14)); *Cl. saccharobutylicum gamma* Izsak and Funk (15); *Cl. saccharo-acetobutylicum*, McCoy (19); *Cl. propyl-butylicum* Mueller and Legg (22) and *Cl. (Bacillus) tetrylium* Owen, Mobley and Aroyo (25) and others which are primarily non or negligible producers of volatile neutral products from starch mashes, clearly place them in the specific group *butyricum* rather than in a doubtful position in the specific group of *Cl. acetobutylicum* as given by BERGEY (1939).

Clostridium acetobutylicum (Weizmann) McCoy. This group which includes the so-called butylic organisms should offer little difficulty to the new-comer to this field.

WEIZMANN (35) originally called his organism *B.granulobacter pectinovorum* but MCCOY et al. (20) and also WEYER and RETTGER (37) renamed the organism *Cl. acetobutylicum* which terminology has since been accepted by all bacteriologists to include those butyric acid producing anaerobes which have a rapid and complete diastatic action on starch mashes producing acetone and butyl alcohol as the main fermentation products. The group is also actively proteolytic and liquefies gelatine. A further differential test is its colonial form on nutrient agar surface. The creamy, compact colonies are removed whole from the surface when picked with a needle. The sporangia are generally elliptical and spores are mainly central (pl. III. fig. 9).

LANGLYKKE, PETERSON and MCCOY (18) observed that on the basis of their cultural behaviour certain organisms designated as *Cl. butylicum* obtained from various collections are strains of *Cl. acetobutylicum*.

Our culture G obviously is a strain of *Cl. acetobutylicum*.

Clostridium pectinovorum Stoermer (Syn. *Plectridium pectinovorum* Stoermer): As already stated, a full discussion on the taxonomy of this group in which we place our cultures A, B and H has been given in a previous publication (36). Strain B is readily distinguishable from the other two strains by its greater production of lactic acid and its small colonial form on nutrient agar and was given the varietal name of "*parvum*". The plectridial form of the sporangia and the ability of the organisms to liquefy gelatine identify them as a group apart from the specific groups *butyricum* and *acetobutylicum*. BERGEY's latest edition of his Manual still names *Plectridium pectinovorum* Stœrmer as a synonym of *Cl. butyricum*, which in view of our knowledge of this species is certainly not justified. Sys-

tematically, this lesser known group appears to be intermediate between the *butyricum* and *acetobutylicum* groups. Similar to *Cl. acetobutylicum* this group liquefies gelatine but, unlike it, produces negligible yields, if any, of acetone and butyl alcohol from maize mashes, in this respect resembling the *butyricum* group.

In conclusion we give the differential tests of the butyric acid producing organisms we have studied and classified.

Clostridium acetobutylicum (Weizmann) McCoy

(1) Very active fermentation activity of maize mash showing rapid, generally complete diastatic activity, "head" formation with slimy strands extending down into the clear serum, which may show yellowish discoloration. (2) High yields of neutral solvents from maize mash. (3) Liquefaction of gelatine. (4) Compact nature of colonies on nutrient agar: whole colony comes away intact from agar surface when fished with needle.

Clostridium butyricum Prazmowski

(1) Non-liquefaction of gelatine. (2) Partial diastic activity (3) Creamy opaque colonies on nutrient agar, later confluent and butyrous. (4) Certain strains produce good yields of acetone and butyl alcohol from sugar mashes.

Cl. butyricum var. *glycerolyticum*

(1) Characteristic stout elliptical sporangia; when young the cell protoplasm is densely granulated but in later stages of development the granulated protoplasm becomes concentrated in one protoplasmic mass and may often be observed rotating fast on its long axis within the sporangium. The mature spore, however, has a thick wall and occupies the full width of the sporangium. Although this characteristic is not exclusive to this strain, yet it appears to be more general here than among our other strains of *butyricum*. (2) Non- liquefaction of gelatine. (3) Actively ferments maize mash, although neutral solvents not produced. (4) Produces appreciable yields of isopropyl alcohol from glucose media. (5) Ferments glycerol.

Cl. butyricum var. *elongatum*

(1) Transulcent, granular, somewhat irregular shaped colonies on nutrient agar slopes. (2) Tendency to produce long slender rods, and very few sporangia on nutrient agar. (3) Non-liquefaction of gelatine. (4) Fair fermenter of glycerol.

Clostridium pectinovorum Stoermer

(1) Crested, often star-shaped, hard, compact form of colony on nutrient agar slopes. (2) Typical capitate sporangia. (3) Liquefaction of gelatine. (4) Partial diastatic activity.

Cl. pectinovorum var. *parvum*

(1) Small, translucent rounded colonies on nutrient agar slopes. (2) Typical capitate sporangia (3) Liquefaction of gelatine. (4) Partial diastatic activity. (5) Produces relatively high yields of lactic acid from glucose media.

SUMMARY

1. An account is given of the isolation of a number of butyric acid-producing anaerobes which were isolated from hemp, jute and flax from Palestine, India and Manila.

2. A brief review is given of the literature dealing with similar organisms obtained from vegetable fibres.

3. Detailed morphological and cultural characteristics of ten of the organisms isolated are given.

4. The organisms ferment maize starch with the production of volatile acids but only one of them shows a rapid and complete diastatic action on starch mashes producing acetone and butyl alcohol as the main fermentation products.

5. Their fermentability of glucose has been studied, and analytical estimations are given of the products of fermentation. The chief end products are neutral volatile products as acetone, butyl alcohol, isopropyl alcohol and ethyl alcohol, and volatile acids as butyric and acetic acids. Lactic and formic acids are also produced, the former in small amounts the latter in minute quantities. Acetyl methyl carbinol is produced by the culture which gave high yields of acetone and butyl alcohol from starch mashes.

6. Tables are given comparing the ability of the organisms to ferment molasses and whey mashes.

7. According to their general morphological characteristics all ten cultures belong to the generic group of *Clostridium*. From their morphological details, cultural characteristics and biochemical activities six of the cultures have been diagnosed as members of the known specific group *Cl. butyricum* Prazmowski and one culture has been found to be identical with another known specific group: *Cl. acetobutylicum* (Weizmann) McCoy. The remaining three cultures are regarded as members of a less known species closely related to, but definitely different from *Cl. butyricum* and *Cl. acetobutylicum*. and named *Cl. pectinovorum* Stœrmer (Syn. *Plect. pectinovorum* Stœrmer).

8. The taxonomy and nomenclature of these organisms are fully discussed. Emphasis is laid on the general acceptance of the term *Cl. butyricum* to cover all strains, including certain species described in the patent literature, which fall within the diagnosis given for the type species *butyricum* in the 1939 edition of BERGEY's Manual of Determinative Bacteriology.

9. Greater use of varietal names is urged indicating as far as possible the minor, easily distinguishable physiological and morphological differences occurring between forms which otherwise fall naturally within one species. Accordingly, for reasons given in the text, three of the six cultures classified as *Cl. butyricum* have been identified as belonging to the type species, two cultures are considered as strains of a varietal form of *butyricum* which has been named variety *elongatum*, and one culture as another varietal form which has been named variety *glycerolyticum*. Likewise one of the three cultures of *Cl. pectinovorum* which differs in certain minor features from the other two is regarded as a distinct variety and has been designated *Cl. pectinovorum* var. *parvum*.

10. In conclusion quick differential tests are given for the diagnosis of the various specific and varietal groups under which the studied butyric acid producing organisms have been placed.

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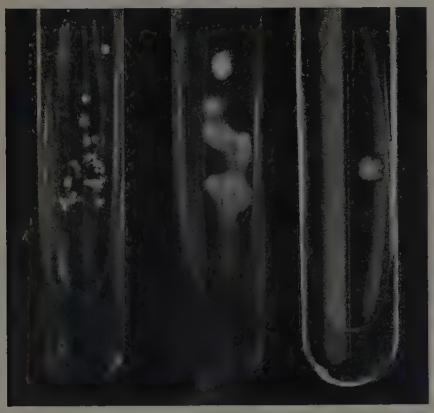

(a) (b) (c)

Fig. 1.

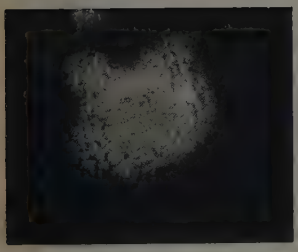

Fig 2.

(a) (b)

Fig. 3.

WEIZMANN & HELLINGER — BUTYRIC ACID PRODUCING ORGANISMS FROM VEGETABLE FIBRES

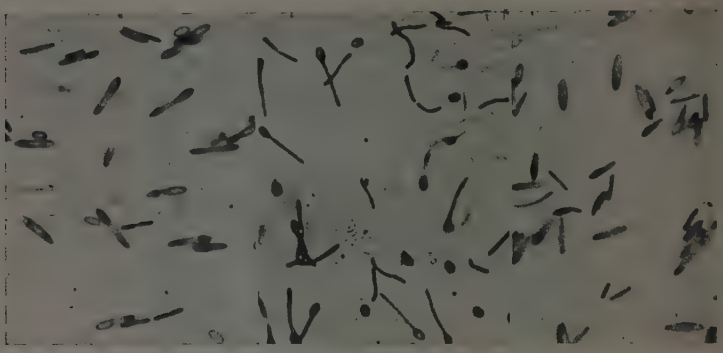

Fig. 5. Fig. 4. Fig. 6.

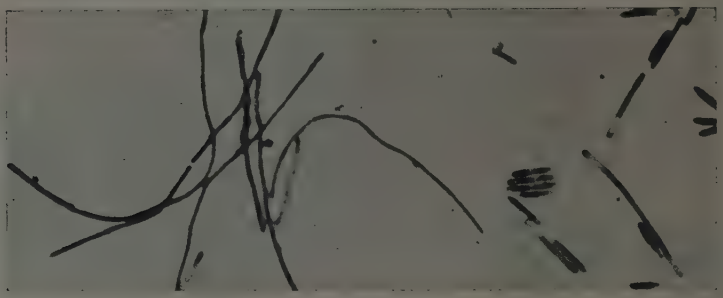

Fig. 7.

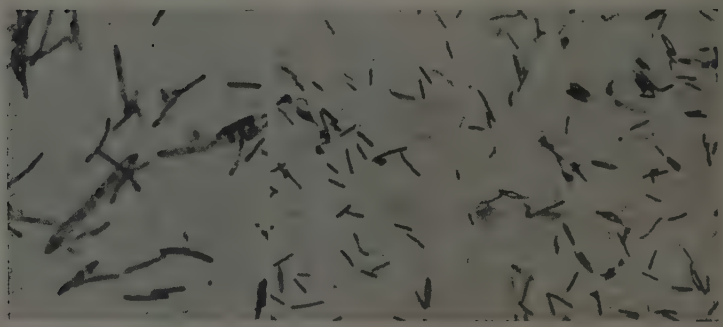

Fig. 8. Fig. 9.

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EXPLANATION OF PLATES

PLATE II.

Figs. 1-2. Typical colonies on yeast-glucose-agar slopes in an aerobic test tubes, kept at 37°C for 48 hours:

Fig. 1. (a) *Clostridium acetobutylicum* (culture G) (x 0.6).
(b) *Cl. butyricum* var. *glycerolyticum* (culture I) (x 0.6).
(c) *Cl. pectinovorum* (culture H) (x 0.6).

Fig. 2. Single colony of *Cl. butyricum* var. *elongatum* (culture E) (x 6).

Fig. 3. (a) *Cl. butyricum* (culture C) and (b) *Cl. acetobutylicum* (culture G) on maize mash at 37°C for 48 hours.

PLATE III.

Figs. 4-7. 48 hours growth on yeast-glucose-agar at 37°C.

Fig. 4. *Cl. pectinovorum* (culture A) (x 1000).

Fig. 5. *Cl. butyricum* (culture C) (x 1000).

Fig. 6. *Cl. butyricum* var. *glycerolyticum* (culture I) (x 1000).

Fig. 7. *Cl. butyricum* var. *elongatum* (culture E.) (x 1310).

Fig. 8. Involution forms of *Cl. butyricum* (culture K) 3 days on 5% maize mash at 37°C (x 1120).

Fig. 9. *Cl. acetobutylicum* (culture G) 5 days on 5% maize mash at 37°C (x 1120).

SCLEROTINIA MINOR ON LETTUCE AND BEANS *)

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(With Plate IV and 1 Text-figure)

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I. INTRODUCTION

Sclerotinia minor was first mentioned in 1902 by SMITH (16) who recorded it as causing a severe attack on hot house lettuce in Massachusetts. SMITH pictured the fungus as greatly resembling *Sclerotinia libertiana*, and differing only in that it had tiny sclerotia. A number of other workers found this fungus on various plants in different parts of America, but the first exact microscopical description was given in 1920 by JAGGER (8) who named it *Sclerotinia minor*. In 1922 BEACH (1) gave a detailed description of lettuce drop caused by *Sclerotinia minor* in Pennsylvania. A celery rot in New Jersey due to this fungus was recorded by POOLE (12) in 1922. WEBER and RAMSEY (18) found, in 1923, that *Sclerotinia minor* was the cause of a tomato stem rot in Florida. In their work on diseases of different varieties of lettuce in Florida, WEBER and FOSTER (17) pointed out that both *Sclerotinia libertiana* and *Sclerotinia minor* attacked not only all varieties of lettuce but also fifty other species of plants. KATA (9), in 1929, isolated *Sclerotinia minor* from the Insect Powder Plant (*?Pyrethrum sp.*) in Japan. The fungus attacked the root collar and leaf stems and caused the entire plant to decay. KATA also succeeded in artificially inoculating lettuce and celery with this organism. *Sclerotinia minor* on lettuce was recorded in Europe for the first time in 1930 when LABROUSSE (10) reported it as occurring in the green houses of Alsace. 75% of the plants were affected by the disease after a sudden cold spell which followed warm weather. FLACHS (5), in 1931, isolated the fungus from diseased lettuce in Bavaria. In 1932 SAVASTANO (15) discovered the fungus in Italy for the first time. He found it attacking lemons and oranges which were lying on the ground in Sicily.

In Palestine, *Sclerotinia minor* was first discovered in 1934 when it was found on a number of plants. In Rehovot it was isolated from the roots of carnations and phlox, from citrus seedlings and from the roots and stems of the potato by various workers of the Division of Plant Pathology; in addition, the author has isolated the fungus from broad beans, lettuce, cabbage, tomatoes and clover. Affected lettuce was also discovered in other localities of Palestine.

Because of the great losses to vegetable growers resulting from this fungus, and the rapidity of its spread by means of sclerotia capable of remaining dormant for a period and then attacking a variety of plants, a further study of the biology and physiology of *Sclerotinia minor* and of the methods of its control seemed warranted.

II. SYMPTOMS

(*a*) *On Lettuce* (*Lactuca sativa*)

The disease first made itself evident at the base of the leaves and progressed along the midrib. The decayed areas turned dark green

and became water-soaked and transparent. In late stages the entire root collar was involved so that the head and roots of the plant were easily pulled apart. White fungal threads and tiny black sclerotia appeared upon the base of the leaves. Longitudinal sections of the stem showed it to contain many sclerotia. The sclerotia were generally found adhering to the roots and rootlets. During heavy attacks all the leaves became water-soaked, and the whole plant finally collapsed and melted into a formless mass of rotten leaf remains and black sclerotia (pl. IV, fig. 1).

The disease was very severe from January to February, 1934. At times the plants were attacked and killed within a few days.

Various varieties of lettuce, including curled lettuce, were attacked by *Sclerotinia minor*.

(*b*) *On Beans* (*Vicia faba*)

The fungus attacked bean plants that had reached a height of half a metre, but had not yet formed pods. The leaves began to turn black at their tips, shrivelled and withered. The stem also blackened. Blackening and shrivelling started at the root collar and then extended upwards. Even when the whole stem was affected, the plant, although withered and dry, remained in an upright position.

III. DESCRIPTION OF THE FUNGUS

The fungus, *Sclerotinia minor*, isolated from lettuce and beans in Palestine, fitted the original description given by JAGGER (8). The sclerotia were black and irregular in shape. They varied in length from 0.5 to 2 mm., often anastomising to form irregular, flattened bodies several millimetres long. The manner of reproduction was purely vegetative. Sclerotia were formed which after a period of dormancy germinated and produced mycelia. No apothecia were formed.

Microconidia, bearing a close resemblance to those of *Botrytis* appeared in December in a number of the test tube cultures on potato-glucose 2%. The black sclerotia formed only along the sides of the tubes. The rest of the colony was covered with a white mycelium upon which were tiny yellow tufts of from six to eight microconidia, 1 mm. in height, which exactly fitted the original description of JAGGER (8). The microconidiophores were short wide mycelial threads measuring 10-15μ x 4-5 μ. They bore a number of round conidia, 3-4μ in diameter. Similar formations were previously found by RAMSEY (13) who believed that they developed under conditions unfavourable to growth. HINO (7) found that microconidia are formed under all conditions. He considered them to be abortive forms of normal macroconidia. As these microconidia are unable to germinate they are of no significance in the life cycle of the fungus. Microconidia were also found by LABROUSSE (10).

In contrast to diseased lettuce, the bean plants dried up instead of becoming water-soaked. Many black sclerotia, ranging in size from one to two mm. could be observed in longitudinal sections of affected bean-stem.

IV. INOCULATION EXPERIMENTS

(*a*) *Laboratory Inoculations*

(1) *Lettuce.* Lettuce was planted in flower pots containing autoclaved soil. When the seedlings had formed three leaves they were thinned out so that only three or four plants were left in each pot. The root collars were then slightly uncovered, and the inoculum, pieces of agar taken from nine day old cultures and bearing *Sclerotinia* mycelium and sclerotia, was placed around them.

The pots were kept in the laboratory at a temperature of 20°C. Four days after the date of inoculation, the leaves of the affected lettuce seedlings drooped and fell off. The basis of the leaves near the root collar, turned a watery brown and was readily torn. Some of the plants showed only marked shrivelling in the area of the root collar. These affected regions later spread, softened, turned black and caused the leaves to drop. The control plants were upright and their roots bore numerous root hairs.

Infection in the laboratory may have developed so rapidly because of the abundant moisture of air and soil and the favourable temperature. As the seedlings were grown in a shaded corner of the laboratory their stems and leaves were long and slender, and this habit may have facilitated disease development.

(2) *Beans.* Potted bean plants growing in sterilized soil were artificially infected and kept in the laboratory at a temperature of 20°C. When the plants were nine centimetres high, the inoculum, pieces of agar containing mycelium and a number of mature sclerotia, was placed around the root collars.

Four days after the inoculation the bean plants in the control pot reached a height of 22 cm. (the plants grew in a shaded corner of the laboratory). The infected plants were only 10-15 cm. high; their root collars were brown and shrivelled and their stems drooped and later collapsed (pl. IV, fig. 2). Longitudinal and cross sections made through the decayed parts revealed that fungal threads grew within the water-conducting vessels, and that the cells surrounding the vessels were black. The stems themselves turned black, the discoloured areas having spread down to the roots and upwards until the whole stem had shrivelled. Tissue cultures made from affected parts developed *Sclerotinia minor* within two days.

(3) *Potatoes.* Attempts to inoculate potato tubers without wounding the tissue proved unsuccessful. In order to facilitate the penetration of the fungus it was necessary to wound the tubers, according

to HORN's method, in the following manner: Cylinders were aseptically removed from the tuber by means of a special boring tool. Pieces of agar, upon which *Sclerotinia* mycelium was growing, were inserted into the hole. The cylinder was then replaced and the outer surface of the wound covered with paraffin to prevent the entrance of saprophytes.

As long as three weeks after inoculation the tubers appeared superficially healthy. Sections revealed that the cylinders were filled with mycelium of *Sclerotinia* bearing many small black sclerotia. The bulk of the tuber tissue remained sound except for the browning of half a centimetre of the tissue surrounding the cylinders. The pectinase enzyme of the fungus is evidently not effective enough to dissolve the middle lamellae of potato cell walls so that *Sclerotinia minor* is unable to penetrate the tuber or spread within its tissues. Potatoes may also contain some substance which hinders the activity of the fungal enzymes as was proved by CHONA (4) in his work on the specialization of parasitism. He found that the fungi *Fusarium fructigenum* and *Botrytis cinerea*, which attack apples, have no effect upon potatoes, whereas *Fusarium caeruleum* and *Pythium sp.*, which are parasites of the potato, are unable to attack apples. CHONA found that potato extract contains certain mineral substances, $MgSO_4$ and KH_2PO_4, which inactivate the pectinase of fungi attacking apples.

(4) *Fruits.* Various species of *Sclerotinia* are known to cause serious diseases of fruits. *Sclerotinia fructigena*, for example, severely attacks apples, pears, plums, etc. REICHERT (14) and others found that *Sclerotinia sclerotiorum* causes fruit rots of the banana and orange in Palestine. *Sclerotinia minor* was found on lemons and oranges in Italy by SAVASTANO (15).

It was therefore of interest to determine whether *Sclerotinia minor* is capable of infecting different kinds of fruit. Experimental inoculations of oranges, clementines, bananas, pears and apples were made with the fungus. The fruit was first washed with alcohol. Then inoculum, in the form of agar covered with mycelium and sclerotia, was placed upon its surface and the fruit was kept in moist chambers under bell jars, at a temperature of 20°C.

(i) *Oranges.* All the inoculations of oranges were successful. A soft, water-soaked, brown region began to form around the inoculum after eight days. The first sclerotia appeared on the tenth day. Their number gradually increased until, two weeks after the date of inoculation, whole fruits had turned brown and were covered with sclerotia borne singly or in groups (pl. IV, fig. 4).

(ii) *Clementine Mandarines.* Five days after the inoculation of clementines a brown, water-soaked region, bearing small colonies of white mycelium appeared around the inoculum. In the course of eight days half of each infected clementine had become brown and watery, and was covered with ripe white sclerotia. Two days later

the entire fruit was covered with small sclerotia, some of which coalesced to form black bodies measuring 0.5 cm. or more in diameter. Fruit inoculated at the stem end did not show the first symptoms of disease until a week after the date of inoculation.

(iii) *Bananas*. On bananas there appeared, thirteen days after inoculation, a brown area, 4 cm. in diameter, where small tufts of mycelium developed and sclerotia began to form. The flesh of affected fruit turned yellowish brown and was penetrated by the characteristic fungal threads. Cultures made from the flesh and peel developed, in the course of three days, colonies of *Sclerotinia minor* bearing white sclerotia.

(iv) *Pears*. A brown, water-soaked region, 3 cm. in diameter appeared on pears, two weeks after inoculation. After three weeks the fruit turned a light brown colour and bore scattered sclerotia. A month after inoculation entire fruits were rotten, brown, watery and covered with a small number of sclerotia. A broad, dark granular mycelium ramified within the flesh of the fruit. Numerous black sclerotia formed in the space between the flesh and peel. These bodies evidently formed only in the presence of air (pl. IV, fig. 4).

(v) *Apples*. Two weeks after apples were inoculated a dry, leathery brown area, 3 cm. in diameter, appeared around the inoculum. Small bunches of white mycelium developed after three weeks. In the course of a month the apples were completely decayed. The peels were brown and on them were a number of clumps of white unripe sclerotia and the flesh was penetrated by the fungal threads.

It is to be remarked that the fungus penetrated the apple with greater difficulty than the orange or clementine. This was probably due to the harder leathery peel of the apple.

(b) *Field Inoculations*

(1) *Lettuce*. In the middle of November lettuce seedlings were transplanted to boxes filled with sterilized sand. As soon as the seedlings had formed three to four leaves their root collars were inoculated with *Sclerotinia* grown on liquid or solid media. One week after inoculation some of the plants showed the first signs of disease. Whereas the control was straight and sturdy and bore five leaves, the infected plants had shrivelled root collars and only three or four drooping leaves, turning black, soft and watery at their bases. The roots and rootlets of inoculated lettuce were not as well developed as those of the control plants.

Three to four weeks after inoculation the number of diseased plants increased. At first the leaves, in particular, were affected, those in contact with the soil turning soft, watery, brown and transparent. White mycelium, bearing numbers of sclerotia grew over the surface of these leaves. Occasionally the infection began at the tip of the leaf and extended down towards the base along the midrib.

In other cases the leaf margin was affected first, turned brown, dried up, and curled. These infections were probably due to infection material splashed from the soil on to the leaves during heavy downpours of rain.

In the normal development of the disease, the infection set in at the root collar and then extended to the leaf bases, turning them a soft, water-soaked brown. The outer leaves turned limp and eventually dropped off. The disease then spread downwards, through the stem, into the root. At this stage many sclerotia were to be found within the roots. Finally, the sclerotia also developed on the rootlets and the whole shapeless mass of soft, water-soaked leaf and stem remains became covered with mycelium and numerous sclerotia.

(2) *Beans.* In January, beans were sown outdoors in boxes containing sterilized sandy loam. When the plants reached a height of 10-12 cm. they were inoculated with mycelium and sclerotia on pieces of agar, placed around the plant at a depth of 1-2 cm. beneath soil level. One row of beans was inoculated by placing the inoculum in a 1 cm. deep groove, at a distance of 2 cm. from the stem. Two weeks later all the plants appeared healthy although the inoculated beans were smaller than the controls, the respective heights being 17-19 cm. and 24 cm. It is to be noted that the controls were erect whereas the infected plants were bent in the middle as a result of unilaterally retarded length growth.

In all the inoculated plants the roots turned black and decayed. In some cases the regions of the root collars were also affected, shrivelled, became watery, and rotted. The roots of the control plants bore many tubercles of nitrogen fixing bacteria. These tubercles did not appear at all on the diseased beans.

In a field inoculation experiment, conducted in the middle of December, the inoculated bean plants died in the course of two weeks. During this period there was heavy rainfall and the temperature was high, averaging 18°-20°C. The root collars of these plants shrivelled and blackened. The black regions extended along the stems, affecting either the leaf bases alone or the entire leaves which wilted and curled. The blackened stems also showed the characteristic drooping. The roots were little affected by the rot.

(3) *Potatoes.* Potato seedlings of 20 cm. height growing in boxes of sterilized soil were inoculated in the beginning of November. In the course of the first month the growth of the plants was normal except for a slight yellowing of the leaves. With the onset of rains, in early December, the leaves touching the ground began to rot and became covered with sclerotia. By December 25th some of the stems hung limply and their root collars were found to be shrivelled and affected by a soft rot. The stems were hollow and filled with black sclerotia which also began to form on the outside of the root collars. The leaves turned black and the whole plants

withered. The rot was concentrated in and above the area of the root collars, descending only a small distance into the root region. The diseased potato plants even yielded a number of tubers.

Summarising the results of the experiments described above we may say that the pathogenicity of *Sclerotinia minor* has been proved by the positive results of field inoculations on lettuce, bean and potato plants (not tubers) and of laboratory inoculations on lettuce and bean plants and on apple, pear, orange, clementine and banana fruits. Potato tubers became infected only after they had been wounded.

V. ENVIRONMENTAL FACTORS IN THEIR RELATION TO GROWTH IN CULTURE

(a) *Temperature*

A number of laboratory experiments was conducted in order to determine the minimum temperatures for the growth of the fungus. Mycelium of a culture originating from a diseased lettuce plant was planted in Petri dishes containing the standard potato-glucose (2%) agar medium. 5-10 cultures were kept at each temperature. Measurements of the extent of growth were taken daily. The size and appearance of the colonies in cultures held at different temperatures, six days after the date of inoculation, are given in Table I.

TABLE I

The rate of growth and formation of sclerotia by Sclerotinia minor at different temperatures

Temperature (°C.)	Average diameter of colonies after six days (mm.)	Development of sclerotia after six days
10	25	none
15	40	none
18	60	slight
20	75	medium
25	85	abundant
30	25	none
32,2	0	none
35	0	none

In the culture kept at 32.2°C the inoculum failed to develop but it remained viable even after two weeks' exposure to this temperature. Inoculum kept at 35°C for five days could not be revived even when transfered to a more suitable temperature.

From this experiment it appears that the optimum temperature for the development of *Sclerotinia minor* is 20°-25°C and the maximum approximately 30°C.

Other workers have arrived at similar conclusions. WEBER and FOSTER (17) found that under natural conditions the fungus is virulent at temperatures aproaching 20°C. FLACHS (5) found the minimum, optimum and maximum temperatures for *Sclerotinia minor* to be 5°C, 20°-23°C, and 35°C, respectively.

(b) *H-ion concentration*

The following experiment was conducted to determine the effect of initial H-ion concentration upon the growth of the fungus:

A liquid medium of potato glucose was prepared and adjusted to the desired pH by the addition of citric acid or calcium carbonate. The fungus was cultured in Erlenmeyers containing 20 cc. of the medium, 5 flasks being used for each level of pH. The flasks were kept at a temperature of 25°C. CLARK's method was used in the determination of the hydrogen ion concentration.

The size and sclerotial formation of the colonies grown on media of varying H-ion concentrations after five days of development are given in Table II.

TABLE II

The rate of growth and formation of sclerotia by Sclerotinia minor on media of different H-ion concentrations

pH	Average diameter of colonies after five days (mm.)	Development of sclerotia after five days
4,2	50	none
5,2	50	none
6,0	60	very slight
6,6	60	medium
7,2	30	none
8,2	30	none

After seven days' growth sclerotia began to form on the media at pH 4.2 and 5.2, while the sclerotia on media kept at pH 6.0 and 6.6 ripened after that period. Only a thin mycelial growth developed on media adjusted to pH 7.2 and 8.2.

The experiment shows that growth of the fungus was poor on very alkaline media and favoured by slightly acid conditions, ranging from pH 6.0 to pH 6.6.

A further experiment was carried out in order to determine whether the fungus increased or decreased the initial H-ion concentrations in the course of its growth.

Liquid potato glucose media of varying H-ion concentrations were prepared in Erlenmeyers. The pH was determined by means

of the colorimetric method. pH readings were taken before and after the growth of the fungus. The measurements presented in table III were made after eight days' growth.

TABLE III

Changes of H-ion concentration induced by Sclerotinia minor after eight days' growth.

Initial pH	Final pH
4.2	3.6
6.0	4.8
7.2	5.8

These figures are typical and show clearly that the fungus secretes acids during its growth.

(c) *Light*

Experiments were conducted to determine whether light hastens or retards germination of the sclerotia of *Sclerotinia minor*. Sclerotia on plants in advanced stages of decay are to be found both in the soil and upon the aerial parts of the plant, open to the sun and light.

Sclerotia were cultured in test tubes containing potato glucose agar. The tubers were kept in the laboratory, some exposed to light and others enclosed in sheaths of black paper.

The sclerotia in the tubes kept in the light were the first to germinate. After six days these tubes were completely filled with mycelium and ripe, black sclerotia, while in the darkened tubes the colonies reached a diameter of only 30 mm and bore only unripe sclerotia formed in the centre of the colonies.

However, after eight days both the cultures in the light and in the dark made practically the same amount of mycelial and sclerotial growth, the only difference being that the sclerotia formed in light were much smaller — 1-2 mm in diameter — than those in the dark which measured 2-4 mm (pl. IV, fig. 3).

Under natural conditions lettuce or potato leaves touching the ground occasionally become infected. Sclerotia develop upon them and germinate, covering the leaf surface with a soft white aerial mycelium.

Hence light does not prevent the germination of sclerotia and may actually hasten it.

VI. SECRETIONS

(a) *Pectinase*

A number of experiments was carried out to determine the enzymes present in the fungus in the course of its development.

Judging from the facility with which the fungus penetrated artificially inoculated fruits, even when they were uninjured, it seemed probable that *Sclerotinia minor* secreted highly active pectinase. Experiments, however, proved that there is little secretion of pectinase.

Thin disks of fresh potatoes, 10μ in thickness, were submersed in a liquid medium upon which the fungus had grown for one week. The disks remained firm even after 36 hours. When the experiment was repeated with a medium upon which the fungus had grown for three weeks, the potato discs became soft after 24 to 30 hours.

These experiments indicate that the exo-enzyme secreted by the fungus in the course of its growth is very weak. The endo-enzyme may be more powerful.

(b) *Toxins*

It seemed likely that the ability of the fungus to penetrate fruit might, also be due to the secretion of some acid. This was proved to be the case with *Sclerotium rolfsii* by HIGGINS (6), who found that this fungus does not penetrate living tissues but first causes the death of the cells by secreting oxalic acid.

SMITH (16), in his study of the parasitism of *Botrytis cinerea* on lettuce found that even before the fungal threads penetrated them, the cells of the host were killed by a thermostable, poisonous substance which he believed to be oxalic acid.

In order to determine whether *Sclerotinia minor* actually secretes toxins such as oxalic acid experiments were conducted to discover the effect on healthy plants of the liquid medium, upon which the fungus had grown.

The fungus was grown in Erlenmeyers containing a liquid medium of potato glucose. When the fungus had grown for two weeks and covered the surface of the liquid, the medium was filtered and poured into test tubes. Sterile liquid media which had not been inoculated were used as controls and poured into other tubes.

Leaves, flowers, or young branches of various plants were placed in the test tubes and the rate of wilting was determined. It was found that there was marked wilting of lupine leaflets after they had stood for only half an hour in the medium upon which the fungus had grown. In the controls wilting was slight and began only after four hours. The stems of lupine placed in the toxic liquid became soft and yellow and died after 24 hours, while the control stems remained normal (Text-fig. 1).

Petunia flowers started to wither after standing in the toxic liquid for ten minutes. After half an hour the limbs curled backwards and shrivelled. In the control wilting began only after four hours.

Clover shoots began to show signs of wilting and the tips of the leaves turned white one hour after they were placed in the toxic medium. In the control wilting set in only after three hours.

The leaves of young myrtle branches began to curl two hours after being placed in the medium upon which the fungus had grown. The leaves in the control remained turgid and normal even after 24 hours.

Text-fig. 1. The effect of toxins secreted by *Sclerotinia minor* on lupine leaves.

All these experiments prove that the fungus, in the course of its growth, secretes toxins into liquid media. These toxins cause plants to wilt by killing their cells. From these tests it appears that *Sclerotinia minor*, just as *Sclerotium rolfsii* in HIGGINS' (6) experiments, kills cells before penetrating them.

In order to discover whether *Sclerotinia minor* causes the death of cells through the secretion of oxalic acid, we further made a number of preliminary qualitative experiments.

The fungus was cultured for two weeks on a medium composed of 100 c.c. water, 1% peptone and 2% maltose. The liquid medium was then examined for the presence of oxalic acid. When calcium chloride was added to a small quantity of the liquid a white precipitate of calcium oxalate formed. This precipitate dissolved upon the addition of hydrochloric acid and again formed when ammonia was added. The controls, the original non-inoculated medium, gave no precipitate. This test showed that the liquid medium upon which the fungus had grown contained oxalic acid.

The test was repeated with a nutrient solution of 1% peptone and 2% levulose upon which the fungus had grown for two weeks. A fair quantity of oxalic acid was found in this medium, while no trace of the acid appeared in the control.

Oxalic acid was also found in a liquid medium containing 3% sucrose upon which the fungus had developed slowly for 2 months.

Other chemical tests were made to verify these reactions. The addition of barium to the liquid medium caused the precipitation of barium oxalate, which dissolved when slightly heated, upon addition of acetic acid.

From these chemical tests it appears that *Sclerotinia minor* secretes oxalic acid. This is probably the toxin which kills the cells of the host plant and makes it possible for the fungus itself to penetrate them.

VII. GROWTH ON VARIOUS NUTRIENT MEDIA AT 20°C.

(*a*) *Solids*

The following is a summary of the growth of the fungus on different solid media.

Potato glucose 2%. — Petri dish cultures became covered with mycelial growth and numerous round, black sclerotia about 2—4 mm in diameter (pl. IV, fig, 5). It is of interest to note that when the fungus was cultured in test tubes containing this medium the growth was often less homogeneous. At times several sclerotia would coalesce to form a black body, one or more centimetres in length. These sclerotial formations generally developed along the sides of the tubes. In some cases the sclerotia formed only at the periphery of the colonies. On potato agar the size of sclerotia was much smaller.

Carrot glucose 2%. — A thin colony, 92 mm. in diameter, containing a few sclerotia, developed after six days' growth in Petri dishes.

Plum glucose 2%. — On this medium growth was slow and sparse. Many tiny (1 mm) sclerotia formed, in particular in the upper part of the tubes.

Cornmeal. — Growth was very slow and sparse, the colonies attaining a diameter of about 65 mm in the course of six days. The branching of mycelial threads was seen clearly, in the microscopic examination. Parallel hyphæ formed short bridges and fused together. At times the ends of the two threads, originating from the same hypha were also seen to fuse. The hyphæ were from five to seven μ in width. Sclerotia appeared only after ten days' growth and averaged 1½ mm in diameter.

Malt. — Growth on malt was rapid and good, attaining a diameter of 95 mm. in ten days. Sclerotia at first formed only at the edges of the colonies but later appeared in the centres as well.

Potato cylinders. — Mycelial growth on sterilized potato cylinders was very poor and thin. White sclerotia began to appear

after five days, turning black on the sixth. Two weeks after inoculation the entire cylinders were consumed by the fungus and nothing but a large number of sclerotia remained.

Carrot cylinders. — Growth resembled that on potato cylinders except that it was slower.

Radish cylinders. — After eight days' growth, the cylinder was covered by a white mycelium bearing a number of scattered sclerotia. Sclerotial formation was much less abundant than on potato cylinders.

Sand saturated with potato extract glucose 2%. — A week after the inoculation the colonies measured 50 mm in diameter. At first the aerial mycelium was loose and sparse, but later, when the sclerotia began to form, it grew more compact and adhered to the substrate. At the end of three weeks many black sclerotia developed. Not a single sclerotium was formed beneath the surface of the medium; a certain amount of air, which is lacking in solid agar preparations, is evidently necessary for sclerotial formation. In the field, under natural conditions, a number of sclerotia were found, even at a depth of 3 cm beneath the surface, when the soil was loose in texture and well aerated.

(*b*) *Liquids.*

Liquid cultures were made in Erlenmeyers.

Potato extract glucose 1%. — A colony, 80 mm in diameter, containing many black sclerotia, covered the surface of the liquid after a week's growth.

Asparagine 1% — glucose 2%. — Growth on this medium was very slow. After eight days, the colonies attained a diameter of only 25 mm, but showed the beginning of sclerotial formation. The fungus continued to grow slowly and formed numerous sclerotia.

Glucose 3%. — Growth was slow and the colonies attained a diameter of 40 mm after two weeks. There was, however, no development above the surface of the liquid. The fungus developed anaerobically within the nutrient solution. After 25 days, the fungus was still at the bottom of the flask and seemed to have stopped growing. There was no evidence of sclerotial formation.

Peptone 1% - levulose 2%. — After 25 days a colony, 60 mm in diameter, had developed with luxuriant aerial hyphæ and the beginnings of sclerotial formations.

Peptone 1% - lactose 2%. — A small colony, only 20 mm in diameter, developed upon the surface of the medium after a week's growth.

VIII. VIABILITY OF SCLEROTIA

(*a*) *Longevity*

In January a number of wilted, blighted bean plants that had been attacked by *Sclerotinia minor* and several lettuce leaves covered by sclerotia were placed in a box containing soil and stored in the laboratory. Every month some of the sclerotia were removed and planted on nutrient media in order to ascertain whether they were still viable.

The following December, almost a year after the diseased material had been gathered, germination of the sclerotia which had been inside the bean stems was almost 100% while that of the sclerotia exposed on the surface of the lettuce leaves was only 50%. These exposed sclerotia may have been more severely affcted by various soil fungi and bacteria.

These experiments showed that most of the sclerotia of *Sclerotinia minor* may retain their power of germination, even after 12 months.

(*b*) *Resistance to heat and drought*

Since the temperature of the soil surface in Palestine may reach very high levels, it was further of interest to determine whether these cause the death of sclerotia.

In order to test the resistance of sclerotia to dry heat, a number of these bodies, free of mycelium, were placed on pieces of glass and heated in thermostats at specific temperatures for definite periods of time. They were then aseptically planted upon potato-dextrose in test tubes and incubated at 25°C.

70°C. — Sclerotia heated to 70°C for 15 and for 30 minutes germinated normally after two days and formed sclerotia after five days, just as did the controls.

80°C. — Sclerotia heated to 80°C. for 10 and for 20 minutes germinated normally and formed numerous sclerotia in the course of a week. Sclerotia exposed to this temperature for 30 minutes germinated later than the controls but nevertheless developed well and formed sclerotia. However, sclerotia, heated to 80°C for 35 minutes were no longer viable.

90°C. — Heating sclerotia to 90°C for 10 minutes did not affect their normal germination. Those heated for 15 minutes were slow to germinate but eventually developed and formed sclerotia. Exposure for 20 minutes prevented germination.

100°C. — Sclerotia heated to 100°C for five minutes germinated five days after inoculation. They developed well and produced new sclerotia. Even after ten minutes' exposure sclerotia still retained their germinability. After being heated for 15 minutes, however, they were no longer viable.

110°C. — Sclerotia heated to 110°C for five minutes were late in germinating, but produced sclerotia abundantly in the course of ten days. Heating the sclerotia to this temperature for ten minutes caused them to lose their ability to germinate.

From the above experiment it is obvious that the sclerotia show a marked resistance to dry heat.

IX. EFFECT OF VARIOUS FUNGICIDES ON GROWTH IN CULTURE

In order to determine the resistance of *Sclerotinia minor* to various disinfectants, the fungus was cultured in Erlenmeyers containing nutrient solutions of potato-glucose and a specific amount of the disinfectant.

Copper sulphate. — On nutrient solutions containing 1/8% of copper sulphate the fungus germinated one week later than in the controls. 14 days after inoculation the colonies were 30 mm in diameter and showed no traces of sclerotial formation, whereas the control cultures attained a diameter of 90 mm and were covered with black sclerotia after nine days' growth. The cultures on the copper sulphate medium nevertheless continued to develop well and formed sclerotia in the course of 2 days' growth.

The fungus did not germinate at all on a medium containing 1/4% copper sulphate, but developed well when transferred to potato glucose.

Formalin. — The fungus developed much more slowly on media containing 1/8°/₀₀ commercial formalin than in the control flasks. The fungus failed to germinate in a 1/4 °/₀₀ solution of formalin but even after a week's exposure to this concentration sclerotia could be induced to germinate by transfer to a normal nutrient medium.

Solbar. — The fungus made almost normal growth when subjected to 1/8% Solbar, covering the solution with its mycelium and sclerotia in the course of two weeks. On a medium containing 1/4% Solbar, germination was delayed for four days, but then the fungus grew well and formed sclerotia.

Uspulun. — In the presence of 1/8°/₀₀ Uspulun the fungus developed normally although it was slow to germinate. There was little growth at 1/4 °/₀₀, only a few immature sclerotia forming at the end of 20 days, and on 1/2°/₀₀ Uspulun germination was inhibited. Upon transfer to a normal medium the fungus developed, although somewhat more slowly than in the controls.

Corrosive Sublimate. — Germination was one week slower than in the control when the fungus was cultured on 1/8 °/₀₀ sublimate. Subsequently, however, growth was vigorous and the culture, in the course of 20 days, was covered by sclerotia. The fungus com-

pletely lost its ability to germinate after being subjected to 1/4‰ sublimate.

Germisan. — Growth was markedly retarded on a medium containing 1/2‰ Germisan. After two weeks the colonies were small, measuring only 20 mm in diameter. The first few sclerotia began to appear on the twentieth day. The fungus did not germiante on 1‰ Germisan but, on transfer to a normal solution, developed slowly and formed sclerotia.

Ceresan. — On 1/8‰ Ceresan growth was almost normal. On 1/4‰, however, germination was greatly delayed and after a week only a thin colony with a diameter of 20 mm developed. There was no growth on 1/2‰ Ceresan, but the sclerotia still retained their viability. The latter was, however, completely lost in the presence of 1‰ of the disinfectant.

These experiments show that *Sclerotinia minor* is quite resistant to the sulphur preparation Solbar at 1/4%, and continues to grow even in the presence of 1/8% copper sulphate.

On the other hand, the fungus is somewhat more susceptible to compounds containing mercury such as corrosive sublimate (1/4‰), Ceresan (1/2‰), Uspulun (1/2‰) and Germisan (1‰). A concentration of 1/4‰ formalin also prevents the growth of *Sclerotinia minor*.

According to the experiments of FLACHS (5), treatment with ten to twenty litres of 1½% formalin or acetic acid per square metre of soil gave good results. Steam disinfection of the soil also proved effective. Various fertilizers had no effect upon the resistance of plants to the disease.

X. SUMMARY

Sclerotinia minor was isolated from soft rot of lettuce and blight of beans in a number of localities in Palestine.

Characteristic sclerotia and microconidia were formed in artificial cultures.

The optimum temperature of the fungus was shown to be 20°-25°C, and the maximum, 30°C.

Good growth was obtained on both acid and alkaline media, though acid media seem to be prefered. The optimum pH is 6—6.6.

The fungus secreted a considerable amount of oxalic acid in the course of its growth.

Germination in light was more rapid than in the dark.

The fungus secreted weak pectinase during its growth.

Good growth was made on a number of natural and synthetic liquid media.

Sclerotia taken from the soil or from diseased plants remained viable over a period of more than one year.

The sclerotia showed a marked resistance to dry heat, germinating even after exposure to 80°C for 30 minutes, 90°C for 20 minutes. 100°C for 10 minutes, and 110°C for five minutes.

Artificial inoculation, both in the field and in the laboratory, of lettuce, beans and potatoes induced the symptoms found in nature. Positive results were also obtained by inoculation, without wounding, of fruits of oranges, clementines, apples, pears and bananas.

Sclerotinia minor appears to be rather more susceptible to mercury compounds than to copper sulphate or to the sulphur preparation Solbar.

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Fig. 1.

Fig 2.

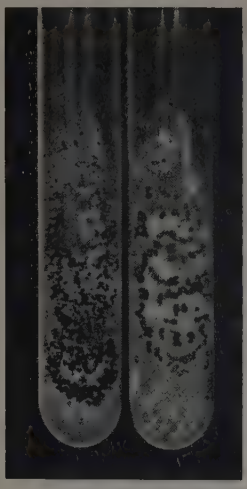

Fig 3.

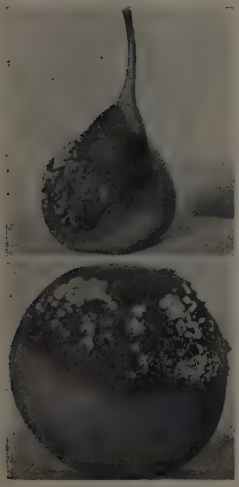

Fig. 4.

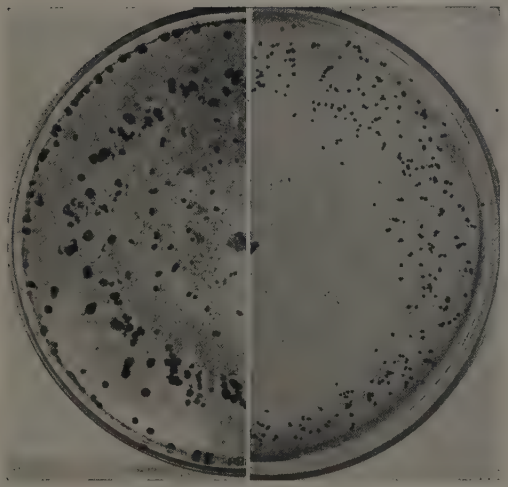

Fig. 5.

SERENI — *SCLEROTINIA MINOR*

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EXPLANATION OF PLATE IV

Fig. 1. Lettuce leaf affected by *Sclerotinia minor.*

Fig. 2. On the left: broad bean seedling inoculated with *S. minor;* growth has been delayed and the root collar has shrivelled.
On the right: control plant.

Fig. 3. The effect of light on sclerotial formation: in the dark (right) the sclerotia formed are larger and fewer in number; in the light (left) sclerotia are more numerous and smaller.

Fig. 4. Below: orange fruit inoculated with *S. minor.* The white bodies are sclerotia in the early stages of their formation.

Above: pear fruit inoculated with *S. minor.*

Fig. 5. On the right: growth of *S. minor* on potato agar; the sclerotia are small.
On the left: growth of *S. minor* on potato glucose; the sclerotia are large.

FIELD TRIALS FOR THE CONTROL OF DOWNY AND POWDERY MILDEW OF CUCUMBERS *)

I. On the Efficacy of various Copper Compounds

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(With 1 Text-figure)

I. INTRODUCTION

Downy mildew (*Peronoplasmopara cubensis* (B.et C.) Clin.) and powdery mildew (*Erysiphe cichoracearum* DC.) annually cause heavy losses to cucumber growers in Palestine. Downy mildew is most destructive on summer and autumn grown crops along the coastal plain and also appears in autumn in the Galilee, in the Valley of Esdrælon, and in the Jordan Valley. Powdery mildew is found throughout Palestine wherever and whenever cucumbers are grown. The control measures usually adopted consist in alternate spraying with 1% Bordeaux mixture against the downy mildew, and sulphur dusting against the powdery mildew. However, spraying with Bordeaux mixture was frequently reported in this country to give unsatisfactory results and failed to increase yields appreciably.

In recent years the injury caused by Bordeaux mixture to tomatoes, cucumbers, and certain other vegetable crops, has formed the subject of numerous investigations; one of the principal results of this work was the proof that the so-called "insoluble" copper

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sprays, such as cuprous oxide and copper oxychloride, injure some copper-sensitive plants far less than Bordeaux mixture. In addition, evidence was forthcoming that the addition of oil to copper sprays may reduce the phytocidal effect of the latter. The primary aim of the trials reported here was therefore to compare the efficacy of Bordeaux mixture with that of various proprietary sprays based on "insoluble" copper compounds, to test these sprays in comparison with some copper dusts, and — in the last trials — to determine the effect of adding oil to copper sprays for the treatment of cucumbers.

We are obliged to the headmaster of Mikve Israel Agricultural School, E. KRAUSE Esq., for permission to use the fields of the school, and to the members of all those settlements, which helped us in the execution of the trials. We further have to thank Messrs. Imperial Chemical Industries (Levant) Ltd., Tel-Aviv, and Messrs. J. Green & Co., Tel-Aviv, as well as the Palestine Vegetable Growers' Association, for the financial support they have given to this work.

Dr. A. BONDI, of the Division of Chemistry at the Agricultural Research Station, Rehovot, was kind enough to advise us on various occasions. Dr. Z. ELAZARI-VOLCANI and Dr. Z. AVIZOHAR have assisted us in the field estimations.

II. MATERIALS AND METHODS

A local selection of cucumbers was used in the trials. Irrigation was by furrows. Dusts were applied while the plants were wet with dew, while sprays were applied only after the dew had dried.

Each replicate plot consisted of 2-6 rows; in order to prevent drift we either did not consider the outmost two rows in the evaluation of results or left 1-2 untreated barrier rows between each couple of plots. The length of rows and size of plots varied and is indicated in the description of each trial. Motor sprayers working at about 25 atmospheres pressure were used in the trials at Ain Shemer, Shfajim, and Negba, while knapsack sprayers were used in all other trials.

The materials tested were:

(1) *Home-made Bordeaux mixture*, prepared from 1 kg. copper sulphate crystals and 660 gr. slaked lime dust in 100 litres solution.

(2) *Flordo Spray*, prepared from 250 gr. copper sulphate crystals, 1 kg. soft soap, and 250 cc. of concentrated ammonium hydroxide (26%) in 100 litres solution (22).

(3) *Perenox*, a proprietary spray based on cuprous oxide, containing not less than 50% metallic copper and 8% chlorine, with a slightly acid reaction; produced by Messrs. Imperial Chemical Industries, England.

(4) *Cuprogreen Concentrated*, a product of Belgium, and *Ob 21*, produced in Germany, both proprietary sprays based on copper oxychloride, containing approximately 50% metallic copper, and neutral in reaction.

(5) The copper dusts *Cupryl, Cuprogreen Dust,* and *Bordeaux Dust Cooper,* all produced in England; the exact composition of these dusts is unknown to us.

(6) *White oil* (medium), produced by Messrs. Shell Company of Palestine, and stated to contain 80% oil, 40-49% distilling over at 636°F, and with 92% unsulphonated residue.

For the control of powdery mildew we further used in the first trials:

(7) *Solbar,* a proprietary spray based on barium polysulphide, produced in Germany.

(8) *Yellow sulphur* (flowers of sulphur).

Yields were recorded as number of fruits per plot rather than their weight. The yield per dunam (1000 square metres) was calculated by multiplying the number of fruits picked with what was found to be their average weight, viz. 87.5 grammes where fruit was picked every second day, and 100 grammes where picked at longer intervals. The number of hills per row was recorded early in each trial and the unit of calculation was the yield per hill. In addition to the size of the yield under each treatment we also calculated its distribution by determining the percentage of fruit yielded at the end (usually the last third) of the picking season. All the yield data were analysed by SAUNDERS' (20) adaptation of the analysis of variance, to the level of P=0.05.

Methods of estimation. — In the earlier experiments (1935-1939) the incidence of downy mildew was roughly estimated according to the relative proportions of green, yellow, and dried leaves on each plot. But in the 1940-41 experiments the incidence of downy and powdery mildew was more accurately estimated by the following scale of categories and marks:

(1) *Downy mildew.*

(a) Leaf free from disease	0 marks
(b) Leaf affected with not more than 2-3 lesions	0.1 "
(c) Leaf affected on not more than 1/3 of its surface	1.0 "
(d) Leaf affected on not more than 2/3 of its surface	2.0 "
(e) Leaf affected on more than 2/3 of its surface and begins to dry up	4.0 "
(f) Leaf drying up partly or completely	8.0 "

(2) *Powdery mildew.*

(a) Leaf free from disease	0 marks
(b) Leaf affected with isolated lesions on not more than 1/10 of the lower surface	0.1 "
(c) Leaf affected on not more than 1/3 of the lower surface	1.0 "
(d) Leaf affected on not more than 2/3 of the lower, or on 1/3 of either surface	2.0 "
(e) Leaf affected on more than 2/3 of the lower, or more than 1/3 of either surface	4.0 "

The total of marks assigned, for each disease separately, to all the leaves examined on a given plot, was divided by the number of leaves examined, and a figure expressing the incidence of each disease (disease rating) was thus obtained for every plot.

The merits and drawbacks inherent in this method of appraising the incidence of disease is being discussed in full by PALTI (17). The greatest obstacle, viz. selecting truly representative samples of leaves of comparable age composition on each plot, was overcome by extracting in each row 10-15 cucumber vines from the maze of intertwined growth and appraising each of their leaves individually.

III. THE TRIALS AND THEIR RESULTS

1935-1939

The first four trials were carried out in 1935-1939 at Mikve Israel and Ben Shemen, in the central coastal plain, on cucumbers sown in spring.

TRIAL NO. 1, 1935. — Treatments were applied on the 1st, 7th, 16th, and 24th of July. Each treatment was replicated 3 times. The results appear in table I.

TRIAL NO. 2, 1937. — Treatments were applied 5 times at intervals of 6-7 days, beginning on 20th May and ending on 16th June. Each treatment was replicated twice, each replicate comprising 65-100 hills. The results appear in table II.

TRIAL NO. 3, 1938. — Treatments were applied 3 times, on 27th June, 13th July, and 26th July. Each treatment was replicated twice, each replicate comprising 90-130 hills. The results appear in table II.

TRIAL NO. 4, 1939. — Treatments were applied 3 times at 3 weeks' intervals, on 26th May, 15th June, and 6th July. Each treatment was applied to a single block comprising 230-330 hills. The results appear in table III.

As the trials nos. 2-4 were directed primarily against downy mildew, all the copper treated plots and the controls were treated 2-3 times with sulphur to prevent the interference of powdery mildew.

RESULTS. — In all these trials downy mildew was absent or appeared too late to cause damage, but powdery mildew was very prevalent. Under these conditions sulphuring alone increased the yield appreciably (table I). Bordeaux mixture, at 2% and 1% strength, on the other hand, gave much lower yields than the sulphur treatment (tables I, II, III) and even reduced the yield slightly below the level of the untreated plots (table I). The copper oxychloride spray Ob 21 resulted in a slight yield increase in the first trial (table I), but somewhat decreased the yield in the third trial (table II). Flordo mixture considerably increased the yield in the third, but slightly decreased it in the fourth trial (tables II, III). Perenox, at 1/2% strength,

reduced the yield in the fourth trial (table III). Of the copper dusts tested, Cupryl somewhat increased the yield in the third trial (table II), while Cuprogreen Dust did not affect the level of yields in the fourth trial (table III).

In the third and fourth trial the fruit was graded (grade A - straight fruit up to 15 cm. in length; grade B - straight fruit of more than 15 cm. length; grade C - blemished or crooked fruit of various sizes) in order to determine the effect of treatments on the quality of the produce. As set out in table IV, the treatments were without effect on the relative percentage of fruits in the various grades. This was confirmed by the application of the chi² test (20).

In the above four trials, in which the number of treatment applications was small, the treatments did not affect the distribution of yield (tables I, II, III).

TABLE I.

Trial No. 1, 1935. The effect of treatments on the size and distribution of yield

Treatment	Block	Yield (in kg.) per plot	Yield (in kg.) mean	% Yield (Control =100%)	% Yield picked at the end of season 14.8. - 27.8.1935 (Total yield per treatment = 100%) per plot	% Yield picked at the end of season 14.8. - 27.8.1935 (Total yield per treatment = 100%) mean
Control	A	129	94	100	20.5	19.47
	B	84			17.0	
	C	69			20.9	
Bordeaux Mixture 2%	A	113	87.3	93	20.0	20.27
	B	67			14.6	
	C	82			26.2	
Ob 21 ½%	A	128	103.0	109	10.1	11.13
	B	97			8.4	
	C	84			14.9	
Yellow Sulphur	A	126	139.0	148	23.5	22.73
	B	147			18.7	
	C	144			26.0	
Solbar 1%	A	115	96.3	102	8.8	14.03
	B	107			12.3	
	C	65			21.0	
Significant difference:			28.29	30.1		4.96

TABLE II.

Trials No. 2 and 3, 1937/38. The effect of treatments on the size and distribution of yield

Treatment	Block	Yield (no. of fruits per hill) per plot	Yield (no. of fruits per hill) mean	Yield in kg. per dunam	% Yield (control = 100%)	% Yield picked during the last two weeks of the picking season (total yield per treatment = 100%) per plot	% Yield picked during the last two weeks of the picking season (total yield per treatment = 100%) mean
Trial No. 2, 1937							
Control (Yellow sulphur)	A	28.3	27.8	6420	100	24.7	22.7
	B	27.2				20.6	
Bordeaux Mixture 1% + Yellow sulphur	A	19.8	18.0	4160	65	22.7	20.2
	B	16.2				17.7	
Solbar 1%	A	27.5	27.3	6300	98	20.9	21.7
	B	27.0				22.4	
Significant difference:			14.37		51.7		10.6
Trial No. 3, 1938							
Control (Yellow sulphur)	A	9.0	8.6	1990	100	33.0	30.6
	B	8.1				28.1	
Bordeaux Mixture 1% + Yellow sulphur	A	6.1	6.8	1570	79	37.5	36.6
	B	7.4				35.7	
Ob 21 1/4% + Yellow sulphur	A	7.8	7.4	1710	86	46.1	40.2
	B	6.9				34.2	
Flordo Spray + Yellow sulphur	A	11.7	12.0	2770	140	41.2	36.0
	B	12.3				30.8	
Cupryl Dust + Yellow sulphur	A	10.8	11.0	2540	128	37.3	41.0
	B	11.1				44.6	
Significant difference:			1.9		22.1		15.06

TABLE III.

Trial No. 4, 1939. The effect of treatments on the size of yield

Treatment	Yield (no. of fruits per hill)	Yield in kg. per dunam	% Yield (Control = 100%)
Control (Yellow sulphur)	26.7	6170	100
Bordeaux Mixture 1% + Yellow sulphur	15.7	3630	59
Perenox ½% + Yellow sulphur	17.9	4130	67
Flordo Spray + Yellow sulphur	22.8	5270	85
Cuprogreen Dust + Yellow sulphur	26.2	6050	98

TABLE IV.

Trials No. 3 and 4, 1938/39. The effect of treatments on the quality of yield

Treatment	Percentage number of fruits in grade				
	A	B	A+B	C	B+C
Trial no. 3, 1938					
Control (Yellow sulphur)	55.2	35.6	90.9	9.1	44.7
Bordeaux Mixture 1% + Yellow sulphur	55.8	34.8	90.6	9.4	44.2
Ob 21¼%+Yellow sulphur	55.2	36.4	91.7	8.3	44.8
Flordo Spray + Yellow sulphur	54.4	34.3	88.7	11.3	45.6
Cupryl Dust +Yellow sulphur	55.1	35.3	90.5	9.5	44.9
Trial no. 4, 1939					
Control (Yellow sulphur)	60.3	29.7	90.0	10.0	39.7
Bordeaux Mixture 1% + Yellow sulphur	61.4	29.7	91.1	8.9	38.6
Perenox ½% + Yellow sulphur	62.7	28.9	91.6	8.4	37.3
Flordo Spray + Yellow sulphur	62.9	27.9	90.8	9.3	37.2
Cuprogreen Dust + Yellow sulphur	61.1	29.3	90.6	9.4	38.9

In view of the fact that in 3 of the 4 trials described above all the plots received regular applications of sulphur dust, we may assume that the incidence of powdery mildew was uniformly slight on all plots. Downy mildew, as mentioned above, appeared only later in the season or not at all. The difference in the level of yield under the treatments tested may thus be ascribed in the first place to the relative phytocidal effects of each treatment. We may therefore draw the following conclusions from these trials:

(1) Copper sprays, especially Bordeaux mixture at 1% strength, are apt to reduce the size of yields (in the absence of downy mildew) below that of plots receiving only sulphur dust treatments.

(2) Flordo spray and copper dusts (Cupryl in 1938, Cuprogreen dust in 1939) do not reduce the size of yield under the same conditions and may even increase it.

(3) None of the treatments affected the grade composition of the yield, and there is therefore no need to record the grades separately in spraying or dusting trials.

1939-1940.

After the phytocidal effect of treatments had been studied in the above trials, two further expriments were arranged in the central

coastal plain, at Mikve Israel, in autumn, when the incidence of downy mildew is usually heavy.

TRIAL NO. 5, 1939. — Treatments were applied 4 times at intervals of 11-13 days on 7th, 18th, and 29th September, and 15th October. Each treatment was applied to a single block comprising 450-600 hills. All the plots were sulphured. The results are summarized in table V.

TABLE V.

Trial No. 5, 1939. *The effect of treatments on the size of yield.*

Treatment	Yield (in no. of fruits per hill)	Yield in kg. per dunam	% Yield (Control=100%)
Control (Yellow sulphur)	1.85	430	100
Bordeaux Mixture 1% + Yellow sulphur	2.89	670	156
Perenox ½% + Yellow sulphur	2.38	550	129
Flordo Spray + Yellow sulphur	2.74	630	148
Cuprogreen Dust + Yellow sulphur	3.03	700	163

RESULTS. — Downy mildew appeared late in September and destroyed all plots within 2-3 weeks. All the treatments checked the disease but slightly, but Bordeaux mixture, Flordo spray, and Cuprogreen Dust increased the yield considerably, while Perenox, at 1/2% strength, resulted in a smaller increase of yield. These results show (a) that treatments at intervals of 11-13 days are unable to check the downy mildew disease effectively, and (b) that a concentration of 1/2% is too strong in the case of Perenox.

TRIAL NO. 6, 1940. — In view of the results obtained in the previous trial, treatments in this trial were applied with much greater frequency. A total of 13 applications was made at intervals of 4 days, beginning on 16th September, 10 days after germination, and ending on 29th October. Flordo spray, the preparation of which is involved while it had proved no more effective than other sprays, was replaced in this trial by Cuprogreen Concentrated. The concentration of Perenox was weakened to 1/3%. Each treatment was replicated twice, but owing to lack of uniformity in irrigation only one block of replicates could be considered in the evaluation of yields.

In this trial the incidence of diseases was for the first time appraised by the scale of categories and marks outlined above. All plots were sulphured uniformly until the date of the first examination (15th October), but sulphuring was discontinued thereafter. The results appear in table VI.

RESULTS. — Bordeaux mixture 1% and Perenox 1/3% proved extremely effective against both the downy and the powdery mildew diseases, reducing infection by more than 90%. Cuprogreen

Concentrated, at 1/3% strength, and Cuprogreen Dust were almost as effective against downy mildew, but markedly less so against powdery mildew. In spite of its fungicidal efficacy, Bordeaux mixture increased the yield only a little, while Perenox and Cuprogreen Concentrated increased it much more, Cuprogreen Dust being intermediate. All the treatments considerably increased the percentage of fruit picked at the end of the picking season.

TABLE VI.

Trial No. 6, 1940. *The effect of treatments on the incidence of downy and powdery mildew and on the size and distribution of yield*

Treatment	Comparative incidence of downy mildew		Comparative incidence of powdery mildew		Yield (no. of fruits per hill)	Yield in kg. per dunam	% Yield (Control yield = 100%)	% Yield picked at the end of season 5-10.11.40 (total yield per treatment = 100 %)
	15.10.	28.10.	18.10.	28.10.				
Control	100	100	100	100	2.02	470	100	10.5
Bordeaux mixture 1%	1	6	4	9	2.53	580	125	20.4
Perenox 1/3%	5	8	4	8	3.25	750	161	22.0
Cuprogreen Concentrated 1/3%	7	18	41	38	3.28	760	162	27.4
Cuprogreen Dust	4	10	30	31	2.79	640	138	25.0

1941

In 1941 the scope of our trials was widened so as to include, besides the central coastal plain, various other centres of cucumber cultivation in the northern and southern part of the coastal plain. We were also able to enlarge the size of each trial, so that each treatment could be replicated 4 times (in No. 11 - 3 times). In view of the results obtained in the preceding year all the 1941 trials were designed to determine the effect of treatments on both downy and powdery mildew, and the plots were therefore not dusted with sulphur.

TRIAL NO. 7. — Cucumbers sown in April were treated with 4 applications at intervals of 6 days, beginning on the date of appearance of downy mildew.

TRIAL NO. 8. — Cucumbers sown in early July were sprayed 9 times at intervals of 4 days, beginning two weeks after germination and before appearance of downy mildew. Each replicate comprised 110-150 hills. The results are summarized in table VII.

Trials Nos. 7 and 8 were carried out in the northern coastal plain of Palestine, at Ain Shemer and Shfajim.

RESULTS. — In trial No. 7, Perenox, at 1/3% strength, again proved much more effective against powdery mildew than Cuprogreen Concentrated (1/3) and than Bordeaux Dust Cooper, which was tested here for the first time. But none of the treatments was satisfactory against downy mildew, although Perenox somewhat delayed the drying up of leaves. In addition, yields were so low - for reasons other than disease and probably due to the local climate - that the treatments had no appreciable effect on the yield.

In trial No. 8, Perenox, at the first estimation, was found to have checked the downy mildew disease more effectively than Cuprogreen Concentrated, but a fortnight later - in spite of 3 applications made in the meantime - the amount of disease on the treatment plots was hardly less than in the control. The treatments increased yields, but not significantly, and were without effect on the distribution of yield.

TABLE VII.

Trial No. 8, 1941. *The effect of treatments on the incidence of downy mildew and on the size and distribution of yield*

Treatment	Block	Comparative incidence of downy mildew		Yield (in no. fruits per hill)		Yield in kg. per dunam	% Yield (control=100%)	% Yield picked at the end of season 25.8—31.8.1941 (total yield per treatment=100%)	
		6.8.41	19.8.41	per plot	mean			per plot	mean
Control	A	100	100	0.62	0.67	160	100	21.5	21.4
	B			0.81				18.6	
	C			0.72				21.7	
	D			0.54				23.9	
Perenox 1/3%	A	57	94	1.22	0.85	200	127	14.4	23.4
	B			0.50				43.8	
	C			0.76				21.4	
	D			0.91				13.6	
Cuprogreen Concentrated 1/3%	A	71	96	1.00	0.95	225	142	18.0	20.4
	B			0.76				24.1	
	C			0.90				24.1	
	D			1.15				20.4	
Significant difference:					0.398		59.4		14.50

The above two experiments may be taken to indicate that, under the conditions prevailing in the northern coastal plain, spraying, even at frequent intervals, will only delay the development of downy mildew to some extent, but will not check the disease. However, even this delay may increase yields by up to 40%.

TRIAL NO. 9. — Cucumbers sown in July were treated 10 times at intervals of 4 days, beginning 12 days after germination and before appearance of downy mildew. Bordeaux Dust Cooper was tested in an additional series of plots treated every 8 days. Each replicate comprised 70-100 hills. The results are set out in table VIII.

TRIAL NO. 10. — Cucumbers sown in July were treated 8 times at intervals of 4 days, beginning 15 days after germination, on the date of appearance of the first symptoms of downy mildew. In this trial the addition of white oil to Perenox was tested for the first time. Each replicate comprised 125-250 hills. The results appear in Table IX.

Trials Nos. 9 and 10 were carried out in the southern coastal plain of Palestine (Gaza district), at B'er Tuvia and Negba.

TRIAL NO. 11. — Cucumbers sown in August were given 14 applications, mostly at 3-4 days' intervals, beginning on the date of appearance of downy mildew, 17 days after germination. Each replicate comprised 100-130 hills. The trial was carried out at Mikve Israel, in the central coastal plain. The results are summarized in table X.

TABLE VIII.

Trial No. 9, 1941. The effect of treatments on the incidence of downy and powdery mildew and on the size and distribution of yield

Treatment	Block	Comparative incidence of downy mildew 14.8.	25.8.	Comparative incidence of powdery mildew 14.8.	Yield (in kg.) per hill per plot	mean	Yield (in kg.) per dunam	% Yield (control yield=100%)	% Yield picked at the end of season 22.8—30.8.1941 (total yield per treatment=100%) per plot	mean
Control	A	100	100	100	0.15	0.33	725	100	7.4	17.7
	B				0.40				18.7	
	C				0.51				26.0	
	D				0.25				18.5	
Bordeaux Dust Cooper at 4 days' intervals	A	55	89	60	0.39	0.34	750	103	22.7	30.7
	B				0.42				27.1	
	C				0.36				30.1	
	D				0.18				42.9	
Bordeaux Dust Cooper at 8 days + intervals	A	90	95	65	0.40	0.33	725	100	23.9	30.3
	B				0.38				28.1	
	C				0.34				27.4	
	D				0.19				41.6	
Perenox ⅓% at 4 days' intervals	A	45	89	15	0.53	0.47	1035	143	32.9	35.0
	B				0.44				29.6	
	C				0.52				36.8	
	D				0.38				40.7	
Significant difference:						0.44		42.4		7.77

RESULTS. — In trials Nos. 9 and 11 the incidence of downy mildew on the control plots was severe, while it was only moderate in trial No. 10.

In trial No. 9 Perenox and Bordeaux Dust Cooper, applied once in 4 days, were, at the first estimation, found to have reduced the incidence of downy mildew, while the dust failed to do so when applied once in 8 days. But 11 days later, at the second estimation, the differences between the control and the treatment plots were small. Powdery mildew was checked effectively only by Perenox. The Bordeaux Dust treatments failed to increase the yield, while Perenox increased it significantly by 43%. All the treatments significantly increased the percentage of fruits picked at the end of the picking season.

TABLE IX.

Trial No. 10, 1941. *The effect of treatments on the incidence of downy and powdery mildew and on the size and distribution of yields*

Treatment	Block	Comparative incidence of downy mildew 25.8.	11.9.	Comparative incidence of powdery mildew 11.9.	Yield (in no. of fruits per hill) per plot	mean	Yield in kg. per dunam	% Yield (control = 100 %)	% Yield picked at the end of season 13.9.1941 (total yield per treatment=100%) per plot	mean
Control	A	100	100	100	1.39	2.22	440	100	12	12
	B				1.85				11	
	C				2.65				9	
	D				2.99				14	
Bordeaux Dust Cooper	A	50	59	107	0.92	1.32	260	60	11	10
	B				0.31				14	
	C				1.95				7	
	D				2.11				8	
Bordeaux Mixture 1%	A	50	89	62	1.43	1.72	340	77	13	13
	B				1.21				17	
	C				1.95				14	
	D				2.30				7	
Perenox 1/3%	A	75	82	40	1.55	2.11	420	95	17	13
	B				1.39				16	
	C				2.45				8	
	D				3.05				12	
Cuprogreen Concentrated 1/3%	A	85	132	59	1.84	2.07	410	93	15	11
	B				1.50				6	
	C				2.40				14	
	D				2,57				10	
Perenox 1/3%+ white oil 1%	A	75	101	24	2.37	2.98	600	134	27	21
	B				1.78				14	
	C				3.10				21	
	D				4.67				22	
Significant difference:						0.50		22.5		5.9

In trial No. 10 downy mildew was obviously not the factor determining the size of yields. The plots suffered from inadequate

irrigation owing to a breakdown in the irrigation arrangements. Under these conditions the only treatment increasing both the size of total yields and of the percentage of yields picked at the end of the season was the combined Perenox and oil spray. The increase was significant as compared with the control as well as compared with the plots sprayed with Perenox alone. Bordeaux Dust Cooper and Bordeaux mixture 1% reduced the yield significantly, while treatment with Perenox or Cuprogreen Concentrated, both at 1/3% strength, did not affect the size of yields.

TABLE X.

Trial No. 11, 1941. *The effect of treatments on the incidence of downy and powdery mildew and on the size and distribution of yields.*

Treatment	Block	Comparative incidence of downy mildew 8.10.	27.10.	Comparative incidence of powdery mildew 8.10.	Yield (in no. of fruits per hill) per plot	mean	Yield in kg. per dunam	% Yield (control = 100 %)	% Yield picked at the end of season 20.10 9.11.41 (total yield per treatment 100%) per plot	mean
Control	A	100	100	100	0.42	2.28	640	100	3.5	5.7
	B				3.78				1.6	
	C				2.63				12.0	
Bordeaux Dust Cooper	A	53	80	59	1.94	2.84	795	125	13.7	17.5
	B				2.39				13.5	
	C				4.29				25.4	
Bordeaux Mixture 1%	A	71	70	36	2.40	2.04	570	89	23.1	24.8
	B				1.08				15.3	
	C				2.63				36.0	
Perenox 1/3%	A	27	49	5	2.70	3.80	1065	167	25.9	27.8
	B				5.52				28.6	
	C				3.19				28.8	
Cuprogreen Concentrated 1/2%	A	73	78	36	1.80	2.97	830	130	22 5	20.2
	B				5.09				19.8	
	C				2.02				18.3	
Perenox 1/3% + white oil 1%	A	37	58	6	4.28	3.32	930	146	37.9	38.5
	B				2.46				35.2	
	C				3.22				42.3	
Significant difference:						2.55		111.8		10.48

In trial No. 11 the estimations of the incidence of diseases proved that Perenox, at 1/3% strength, with or without the addition of oil, was definitely the most effective fungicide both against downy and powdery mildew, Bordeaux mixture 1%, Cuprogreen Concentrated (1/2%), and Bordeaux Dust Copper being less effective. Bordeaux mixture slightly reduced the yield, Perenox, with or without oil, increased it considerably, while Cuprogreen Concentrated and Bordeaux

Dust Cooper were intermediate. All treatments significantly increased the percentage of yield at the end of the picking season, and the combined Perenox and oil spray resulted in a further significant increase of this percentage beyond that caused by the other treatment (text-fig. 1).

Text-Fig. 1.
Trial No. 11, Mikve Israel.
On the right:
untreated control row — all the leaves have dried up owing to the attack of downy mildew.
On the left:
row sprayed with Perenox ⅓% — the plants are still covered fairly well with leaves.
(photographed 2—3 weeks after the control plots had ceased to yield fruit).

IV. DISCUSSION

A. *Fungicidal action of copper treatments on downy & powdery mildew*

1. Downy mildew. The efficacy of Bordeaux mixture against downy mildew has long been known (2), but authors differ in the concentrations they recommend from 0.5 to 1.5%. In our experiments Bordeaux mixture at 1% strength was effective; higher concentrations are out of the question under the warm and dry conditions prevailing in Palestine (cf. below), but lower concentrations still remain to be tested.

There appears to be little literature on the effect of the insoluble coppers on downy mildew. Mahoney & Stier (13) used cuprous oxide for the control of this disease on melons. Horsfall and his co-workers (9) investigated the effect of these sprays on the cucumber plants only. We found proprietary products based on copper oxychloride (Cuprogreen Concentrated) and on cuprous oxide (Perenox) to be effective against this disease. Especially the latter gave excellent results, while the former was somewhat weaker in action.

Copper dusts were found by Weber (25) to have a lower fungicidal effect on downy mildew than Bordeaux mixture. These findings were confirmed by our trials, where none of the dusts tested equalled Bordeaux mixture or Perenox in their action on downy mildew.

2. Powdery mildew. The controlling effect of Bordeaux mixture, ranging from 1/4% to 1 1/2% in strength, has variously been mentioned during recent years (6, 10, 11, 16, 18). In our trials this effect became evident as soon as spraying intervals were reduced to only a few days. All copper fungicides were seen to have some action on powdery mildew, but they differed greatly in the measure of control they afforded. Perenox was by far the most effective, and almost entirely suppressed powdery mildew development. Cuprogreen Concentration and Bordeaux mixture were moderately effective in most trials, and only the copper dusts had little effect on powdery mildew. These results agree with the findings of Miller & Barrett (14) on melons, who state that copper dusts do not control powdery mildew.

3. Combined control of the two mildews. Guba (6) was the first to recommend the combined control of downy and powdery mildew by Bordeaux mixture. Our trials confirm that Bordeaux mixture may be used for this purpose but show in addition that the two diseases may be controlled even more effectively by the insoluble copper spray Perenox.

B. *Effect of treatments on the size of yield and on the duration of the period of fruiting*

1. Size of yield.

The effect of treatments on the size of yield represents the balance of their fungicidal and phytocidal effects. The latter, however, cannot be measured directly and, as pointed out by Palti (17), can only be assessed indirectly by careful comparison of the fungicidal effect of each treatment with its effect on the size of yield.

(a) bordeaux mixture. At 1% strength, Bordeaux mixture proved an effective fungicide but failed to increase the yield of cucumbers or even reduced it, whereever the downy mildew appeared late or not at all, or where the plants suffered from drought. The treatment may therefore be considered to have affected the cucumber leaves adversely.

According to the researches of Horsfall and his co-workers (8,9), Bordeaux injury to tomatoes and cucumbers is caused by the fact that the cuticle is injured by alkaline solutions and transpiration is thus increased. In addition, the lime penetrates and hardens the tissues. This injury appears to be restricted to young plants (5,8). In our trials spraying commenced when the plants were still very young and sprays were directed to the lower side of the leaves, where the cuticle is much more delicate than on the upper side. In addition applications were frequent and the spray load very heavy. We may, therefore assume that the yield reduction caused by treatment with Bordeaux mixture, as apparent in many of our trials, was due to excessive transpiration. This assumption is supported by the fact

that Bordeaux injury was particularly marked where the plants grew under conditions of insufficient water supply (table IX). This agrees with the findings of WILSON & RUNNELS (19, 26, 28) and of other writers (1,15), who have stressed the fact that Bordeaux injury on various plants is most prominent under drought conditions.

(b) INSOLUBLE COPPER SPRAYS. Perenox proved to be the treatment with the highest fungicidal efficacy. At 1/2% strength, this spray reduced the yield no less than Bordeaux mixture, but at 1/3% strength Perenox greatly increased the yield. Cuprogreen Concentrated, at 1/3-1/2% strength, was weaker in its fungicidal action, but nevertheless equalled Perenox in its effect on the yield, so that the difference in yield between these treatments was never significant. The fact that Perenox, a more active fungicide than Cuprogreen Concentrated, failed to increase the yield more than the latter, indicates that even at 1/3% strength Perenox somewhat affected the cucumber leaves; some yellowing of the leaf margins was in fact observed on all plots treated with this spray, and a further reduction of the concentration of this spray may thus give even larger yields. Cuprogreen Concentrated appears to be of low phytocidal activity.

The insoluble copper sprays have recently been proved to be less injurious to cucumbers and tomatoes than Bordeaux mixture (9,30). This is confirmed by our trials, in which copper oxychloride and cuprous oxide sprays increased the yield, where Bordeaux mixture failed to do so, and did not reduce the yield, in the absence of disease, where Bordeaux mixture reduced it. The yellowing of leaf margins observed in our trials on the plots sprayed with Perenox, has not been mentioned by HORSFALL, HERVEY & SUIT (9), who investigated the effect of pure cuprous oxide spray on cucumbers.

(c) COPPER DUSTS. All the dusts tested were only moderately effective fungicides. Their effect on the yield was not uniform. Cupryl and Cuprogreen Dust, in the earlier trials, gave higher yields than most sprays where the downy mildew was not prominent, and these dusts appeared to be harmless to the leaves. However, Bordeaux Dust Cooper, tested in the 1941 trials, nowhere increased the yield, but reduced it significantly under drought conditions (table IX), and thus seemed to have some adverse effect on the leaves.

Various authors have found the use of copper dusts on cucurbits to be more beneficial than Bordeaux spraying (23). HORSFALL, HERVEY & SUIT (9) have explained the advantage of the dusts over the sprays by pointing out that the contact made by the dusts with the leaf surface is not as close as that made by sprays, and that the former are therefore less likely to injure the cuticle. The absence of harmful phytocidal effects in most copper dusts may thus in some cases be imagined to compensate for their generally weaker fungicidal action.

(d) Flordo spray. This spray was used in 1938/39 in two trials in which the downy mildew disease did not appear. In both cases the yields of the Flordo plots exceeded those of the plots sprayed with other preparations. This may be due to (a) the much lower copper content of the Flordo spray, amounting to only one quarter of that of 1% Bordeaux mixture; (b) the possible formation, by the soap included in the spray, of a protective colloid preventing the penetration of copper into the tissues; and (c) the possible formation of copper salts of the fatty acids in the soap which would also prevent deep penetration of the copper. But in spite of its low phytocidal activity the chances for this spray to become widely accepted are small, as its preparation is rather too complicated.

(e) White oil. The addition of medium white oil to the Perenox spray had no marked effect on the fungicidal properties of the latter. But in one trial, under drought conditions, the combined Perenox and oil spray resulted in a large yield increase, while the plots sprayed with Perenox alone did not outyield the control (table IX). But in another trial, under normal moisture conditions, the addition of oil failed to increase the yield beyond that of the plots sprayed with Perenox alone (table X).

Horsfall & Harrison (8) have recommended the use of cottonseed oil for the reduction of copper damage on tomatoes and explain the action of the oil as preventing the transpirational increase occasioned by copper sprays alone. Other authors successfully used the same oil on cucumbers (31) and mineral oils on tomatoes (1, 29, 30) for the same purpose. In the light of these findings, our results may be interpreted as showing (a) that under drought conditions the copper sprays probably accentuated the drought injury, and their beneficial effect in checking the downy mildew was thus obliterated: the copper-oil spray, however, did not apparently increase the drought effect and thus increased the yield; (b) under normal moisture conditions no additional advantage derived from the conservation of moisture by the oil, and the latter thus had no marked effect on the size of yield.

2. Duration of fruiting period.

The copper treatments usually not only increased the size of yield, but also extended the duration of the fruiting period. In those of our trials, in which treatments were applied at frequent intervals, the proportion of fruits picked at the end of the season was thus greatly increased. This effect was, of course, most prominent in the case of treatments which gave large increases in the size of yields, as these increases were often due not so much to larger yields during the normal picking period as to an extension of the latter. In fact, the increase of yield at the end of the picking period was always relatively much larger than the total yields. Thus Perenox in one trial (table X) increased total yields by 67%, as compared with the con-

trol, while yields at the end of the season were increased from 6% in the control to 47%, i. e. almost eightfold. But the effect of copper treatments in extending the fruiting period was noticeable even where the treatments did not increase the size of total yields such as in the case of Bordeaux Dust Cooper (table VIII) and of Bordeaux mixture (table X) in two of the trials.

A special effect on the duration of the fruiting season appears to have been exerted by the white oil spray, which was tested in two trials. In one case, under drought conditions, where only this spray increased yields, it also was the only treatment to prolong the picking period (table IX). But even greater interest attaches to the fact that in the second case, where Perenox alone yielded some 20% more than the Perenox-oil spray, the latter significantly exceeded the former in the percentage of fruit picked during the last third of the picking period. This extension of the fruiting period by the copper, and especially by the oil treatments, is of greatest practical importance, as prices rise sharply towards the end of the season. As far as we are aware, this effect of the oil spray has not previously been mentioned in literature.

C. *Intervals between applications and profitability of treatments*

1. Intervals.

As apparent from the above results, the length of intervals between successive applications is one of the primary factors determining the success of mildew control in Palestine. In late summer, conditions are so favourable for downy mildew development, that applications at 10-14 days' intervals are inadequate and even applications at 4 days' intervals do not always succeed in delaying disease development for more than a brief period.

Similar conclusions have been reached by Doran (4), who recommends 10 applications in the course of the season, and by Clayton (3), who states that under Long Island conditions cucumbers should be sprayed twice a week. Although the exact number of applications necessary in Palestine cannot yet be stated, as it largely depends on the very varying local conditions, it may be assumed that adequate control of downy mildew necessitates at least 8 applications during the season.

2. Profitability.

Comparing the expenses involved in spraying and dusting, spraying is seen to require much more labour, while dusting necessitates a considerably larger outlay for fungicidal material. As the cost of the latter is constant throughout the country, but the cost of labour varies greatly in different localities, the preferability of one mode of treatment to the other depends chiefly on the local cost of labour. Where labour is cheap, spraying will be cheaper than dusting in full-grown fields requiring the application of large quan-

tities of fungicides per dunam. However, in young cucumber fields, where the amount of material required is still small, dusting may be cheaper in the first few applications. Considering, moreover, that young plants are particularly susceptible to copper damage, and dusts generally appear safer than sprays, this practice may be advantageous from more than one point of view.

The increase in yields obtained in our experiments under conditions favourable to downy mildew development ranged from 30 to 70%. Whether or not such increases will cover the spraying or dusting expenses obviously depends on the level of yields. Where yields are very low, as in our trials in the northern coastal plain, this will not be the case. But wherever the yields of untreated fields approximate at least 500 kg. per dunam, as in our 1940/41 experiments in the central and southern coastal plain, treatments will cover their expenses and provide for additional profit.

When calculating the profitability of treatments, due regard must also be had to the extension of the picking period by the treatments. This factor is of outstanding economical importance with cucumbers grown in late summer, and may then increase profits far more than would appear from the increase in the size of yields alone.

V. SUMMARY

(1) Eleven spraying and dusting trials for the control of downy mildew (*Peronoplasmopara cubensis*) and powdery mildew (*Erysiphe cichoracearum*) of cucumbers have been carried out in various parts of the coastal plain of Palestine over a period of 6 years (1935-1941) with the use of various copper compounds.

(2) The frequency of applications was found to determine largely the success of treatments: applications at 10 days' intervals proved ineffective against downy mildew, while several materials succeeded in controlling the disease when applied at 4 days' intervals.

(3) Best control of downy mildew by frequent applications was given by the Perenox spray, based on cuprous oxide, at 1/3% strength. Bordeaux mixture (1%), the proprietary copper oxychloride spray Cuprogreen Concentrated (1/3-1/2%), and various copper dusts were somewhat weaker in their action.

(4) Of all the copper materials tested, only Perenox controlled powdery mildew satisfactorily.

(5) Bordeaux mixture (1%) failed to increase, or even reduced, the yield and was inferred to be injurious to cucumber foliage. Perenox, at 1/3% strength, caused the leaf margins to turn yellow, while Cuprogreen Concentrated (1/3-1/2%) did not affect the leaves; the two sprays equalled one another, however, in their effect on the

yield, which they increased by 30-70%. The effect of the copper dusts on the yield was not uniform.

(6) The addition of medium white oil (1%) to a 1/3% Perenox spray increased the yield by 35% in one trial under drought conditions, but failed to increase the yield in a second trial under normal moisture conditions.

(7) The copper treatments, and especially the Perenox-oil spray, markedly increased the percentage of fruit picked during the last part of the fruiting period, and thus extended the duration of the latter.

(8) The treatments were without effect on the relative proportion of grade A, grade B, and grade C fruit yielded by the cucumbers.

(9) Spraying is cheaper than dusting, where labour is obtainable at low cost. But in young fields, where the quantities of dust required are still small, and which are particularly susceptible to spray injury, dusting may be preferable.

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FIELD TRIALS FOR THE CONTROL OF TOMATO LEAF DISEASES *)

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I. INTRODUCTION

Tomato crops grown in Palestine suffer mainly from the following five leaf diseases: powdery mildew (*Leveillula* (*Oidiopsis*) *taurica* (Lev.) Arn.), leaf mould (*Cladosporium fulvum* Cke.), *Alternaria* blight (*Alternaria solani* Ell. et Hart.), *Phytophtora* blight (*Phytophthora infestans* (Mont.) de By.) and *Septoria* leaf spot (*Septoria lycopersici* Speg.). Powdery mildew and *Alternaria* blight occur in all parts of the country and and at all seasons, while leaf mould chiefly attacks autumn-grown tomatoes in the coastal plain and winter or spring grown crops in the Jordan Valley and the Valley of Esdrælon. In these valleys winter crops may suffer severely from *Phytophthora* blight. *Septoria* leaf spot is found in various parts of Palestine, mostly in spring or summer, but occasionally also in winter.

The 5 trials described here were designed to test various means of combating the above diseases under the very varying conditions prevailing in the different parts of Palestine. They were therefore carried out in the moderately warm and very humid coastal plain (at Mikve Israel, near Tel Aviv), in the hot and moderately humid Jordan Valley (at Degania, near the exit of the river Jordan from the Sea of Tiberias), and in the warm and relatively dry eastern part of the Valley of Esdrælon (Nir David).

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Phytophthora blight and *Septoria* leaf spot failed to appear in any of the trials and *Alternaria* blight, against which the trials in the valleys were chiefly directed, was only of secondary importance, so that we were unable to test control measures against these three diseases. Powdery mildew, on the other hand, strongly attacked the 1941 trial in the Jordan Valley, and leaf mould the 3 trials carried out in the coastal plain. Our results consequently deal mainly with the effect of fungicides on the latter two diseases and with their phytocidal effect on tomato plants. In addition, extensive frost injury occurring in one of the trials enabled us to observe the effect of various sprays on the extent of this injury to tomato leaves and fruits.

The materials tested included lime-sulphur sprays, Bordeaux mixture, so-called "insoluble" copper sprays, shirlan paste, and lastly mineral oil which has lately been reported to reduce copper-spray injury to tomatoes.

We wish to thank the headmaster of Mikve Israel Agricultural School, E. KRAUSE Esq., for permission to use the fields of the school, and the members of Degania and Nir David collective settlements for their help in the execution of the trials. We are further obliged to Dr. F. LITTAUER and Dr. Z. AVISOHAR, of the Division of Plant Pathology, for their assistance.

II. MATERIALS AND METHODS

All experimental plots were irrigated by furrows.

The sprays were applied by means of knapsack-sprayers working at 4-10 atmospheres pressure; but a motor-sprayer (25 atmospheres) was used at Nir David. The sprays were applied only after the dew had dried on the leaves.

The plants were spaced 30 cm. apart in the rows, with 1.25 m. between the rows, where the varieties Marmande and Tezier Prime were used, but with only 85 cm. between the rows in the case of the Stoffert's Immune variety. The plants were trained as cordons.

Each replicate plot consisted of 4-5 rows, the two outer rows serving as barriers to prevent drift, and only the central rows being considered in our results. At Nir David, where high spraying pressures were used, there were 4 barrier rows between each pair of plots.

The following materials were tested:

(1) *Sulfinette,* a proprietary lime-sulphur spray of 33° Baumé, produced by Messrs. Cooper McDougall Ltd., England.

(2) *Cita Lime-Sulphur Spray,* 30-32° Baumé; produced by Messrs. Chemical Industries Tel Aviv Ltd., Palestine.

(3) *Sulfocide,* a proprietary sodium pentasulphide spray, produced in the United States.

(4) *Home-made Bordeaux Mixture,* prepared from 1 kg. copper sulphate crystals and 660 gr. slaked lime dust in 100 litres solution.

(5) *Flordo Spray,* prepared from 250 gr. copper sulphate crystals, 1 kg. soft soap, and 250 cc. of concentrated ammonium hydroxide (26%) in 100 litres solution (19).

(6) *Perenox,* a proprietary spray based on cuprous oxide, containing not less than 50% metallic copper and 8% chlorine, with a slightly acid reaction; produced by Messrs. Imperial Chemical Industries, England.

(7) *Cuprogreen Concentrated,* a proprietary spray based on copper oxychloride, containing approximately 50% metallic copper, and neutral in reaction; a product of Belgium.

(8) *White Oil* (medium), containing, according to makers' specifications, 80% oil, 40-49% distilling over at 636°F and with 92% unsulphonated residue; a product of the Shell Company of Palestine.

(9) *Alboleum Spray Oil,* produced by Messrs. Imperial Chemical Industries, England. Specifications of the properties of this oil were not available.

(10) *Shirlan AG,* a proprietary paste based on salicylanilide, produced by Messrs. Imperial Chemical Industries, England.

Methods of estimation.

For the purpose of estimating the effect of treatments on the incidence of powdery mildew and of leaf mould we distinguished between the basal, central, and top parts of the plants, and estimated each part separately. In view of the fact that the leaves of the basal part were frequently found to dry up, for reasons other than leaf diseases, difficulties were encountered in estimating this part accurately. In evaluating our results we therefore considered only the estimates of the central and top parts. Only in one trial (Mikve Israel, 1941) the estimation was made while the plants were still young and in this case it was found possible to estimate accurately even the basal part of the plants.

The method of estimation resembled that recently described by us for the estimation of cucumber mildews (16). The following categories and scale of marks were used to denote separately the extent of infection by powdery mildew and leaf mould:

(a) free from disease	0 marks
(b) few spots on a few leaves	0.1 marks
(c) most leaves affected on not more than half their surface	1.0 mark
(d) most leaves affected on more than half their surface, but not yet drying up	2.0 marks
(e) numerous leaves begin to dry up	4.0 marks
(f) most leaves dried up	8.0 marks

The sum of marks assigned to the various parts of the plants was taken as an index of the incidence of each disease (disease rating), and the average of marks of the replicates of one treatment was held to indicate the fungicidal efficacy of the treatment. For the sake of easier comparison the average marks of the plots under each

treatment were expressed as percentage of the average marks assigned to the control plots.

The incidence of frost injury to the leaves in the Nir David trial was estimated on the following scale of marks:

(a) the majority of the lower, and part of the upper leaves have remained green and turgid ..... 1 mark
(b) only part of the lower leaves is still green and not more than slightly wilted ..... 2 marks
(c) all the upper and most of the lower leaves have wilted and dried up ..... 3 marks
(d) all leaves have dried up ..... 4 marks

Yield Records. — The yield was recorded in kg. per plot. At Nir David, where the fruits suffered frost injury, the number of fruits so affected was recorded at each picking.

Evaluation of results. — The significance of differences between the results obtained with the treatments tested was determined by the analysis of variance method as adapted by SAUNDERS (17) for field experiments. In addition to the total yield we also considered the effect of treatments on the distribution of yields during the picking period. For this purpose we calculated the percentage of fruit yielded under each treatment at the end of the picking period (usually its last third). The effect of treatments on these percentages, and in Nir David on the number of fruits injured by frost, was also examined by the above method of statistical analysis. All the calculations of significant differences were made to a level of probability of 1:20 (P=0.05).

III. THE TRIALS AND THEIR RESULTS 1939 — 1940

Trial No. 1, Mikve Israel.

In this trial on a plot of the Marmande variety planted in June 1939, the materials tested included home-made Bordeaux mixture 1%, Perenox 0.5%, Flordo Spray, Sulfocide 0.5%, and yellow sulphur. 5 applications of these treatments were made at 3 weeks' intervals commencing before appearance of any disease symptoms, on July 6th, and ending on September 26th.

RESULTS. — The experimental plots were strongly attacked by leaf mould and none of the treatments succeeded in controlling the disease. The trial thus clearly demonstrated that treatment applications at intervals as long as 3 weeks are entirely inadequate for the control of leaf mould.

Trial No. 2, Mikve Israel.

In view of these results of the first trial, treatments in the 1940 trial were applied at much more frequent intervals but we were so limited in space that not all the treatments could be tested again. Besides the copper and sulphur sprays, Shirlan paste was included in this trial.

Seedlings of the Stofferts Immune variety were planted out on September 6th on light-medium soil. The programme of treatments was as follows:

(a) Control
(b) Perenox 0.5% + Alboleum 1%
(c) Shirlan 0.5%
(d) Cita Lime-Sulphur Spray 1.5%
(e) Sulfocide 3/4% + Soft soap 3/8%

Each treatment was replicated twice, each replicate covering approximately 45 square metres (without barrier rows). Spraying commenced on October 14th, 4 days after leaf mould had first been recorded, and was first continued in 7 applications at 3-5 days intervals (until November 10th) and then in a further 8 applications at 5-7 days' intervals (up to December 30th). At the first two applications all sprays were used at only three quarters of the strength stated in the programme. The rate of spraying was 240-280 litres per dunam at the first 8, but only 140 litres at the last 8 applications, as a finer nozzle was then used and the plants had meanwhile been tied to the cordons. The fruits were picked at 2-4 days' intervals between November 19th 1940 and January 16th 1941.

RESULTS. — The first disease to appear on the experimental plots early in October was powdery mildew. However, this disease developed slowly, and the leaf mould disease, first recorded on October 10th 1940, spread so much faster that it obliterated the symptoms of mildew.

The estimations were therefore limited to the determination of the incidence of leaf mould under the various treatments. The dates and results of the three estimations are detailed in table I.

At the last estimation the tomato plants on the control plots were found to have almost entirely dried up owing to leaf mould attack. The table indicates that all the treatments reduced the incidence of disease by at least 70%. On all the treatment plots leaf mould infection on the date of this estimation was but slight to moderate and the plants were still well covered with green leaves. The treatments did not differ markedly from one another in their effect on leaf mould.

The effect of treatments on the size of yields and on their distribution over the picking period is apparent from table I. The yields obtained from two replicate plots are seen to agree closely in the case of the Perenox + Alboleum and of the Cita Lime-Sulphur treatments. The latter two sprays increased yields by 25-35%, but owing to the low number of replicates this increase was not statistically significant. The yields of the Shirlan AG and Sulfocide plots varied considerably in the two replicates, and no conclusions as to the effect of these sprays on the size of yields can be drawn from these results. All the treatments, with the exception of Sulfocide, significantly increased the percentage of fruit picked at the end of the picking period.

TABLE I.

Effect of Treatments on the Incidence of Leaf Mould and on the Size and Distribution of Yields

Treatment	Comparative incidence of leaf mould (average of 2 plots)			Yield in kg.			% Yield (Control = 100%)	% Yield at the end of the picking season 30.12.40—16.1.41 (total yield per treatment=100%)	
	1.11.40	27.11.40	18.12.40	per plot	mean	per dunam		per plot	mean
Control	3.3	23.3	100	52.0 54.9	53.45	1425	100	5.0 10.2	7.6
Perenox 0.5% + Alboleum 1%	2.4	4.1	13.2	75.6 68.4	72.00	1920	135	29.6 30.4	30.0
Shirlan AG 0.5%	1.0	5.1	11.5	48.9 72.1	60.50	1610	113	20.0 30.2	25.6
Cita Lime-Sulphur Spray 1.5%	0.0	1.9	18.4	66.5 68.7	67.60	1800	126	25.3 27.8	26.6
Sulfocide 0.75%	0.4	2.2	29.5	66.4 46.2	56.30	1500	105	21.5 11.5	16.5
Significant difference:					31.3	825	57.9		14.63

1941

Basing on the results of the two preliminary trials outlined above, the scope of our experiments was widened in 1941 to include trials in the Jordan Valley and the Valley of Esdrælon, as well as at Mikve Israel, and each of the treatments tested was applied to 4 replicate plots.

The trial at Mikve Israel was again chiefly directed to the control of leaf mould and the two treatments most successful in the pre-year, Cita Lime Sulphur spray, and Perenox + oil, were recapitulated. As Alboleum was not available at the time, it was replaced by Shell medium oil. We further tested another lime-sulphur preparation and the copper spray most commonly used in Palestine, home-made Bordeaux mixture of 1% strength. Shirlan was not retested as it was far more expensive than the other sprays, but did not exceed them in efficacy in the 1940 trial.

The trials in the valleys were primarily directed against *Alternaria* blight and only in the second place against powdery mildew. They therefore included a number of copper sprays but only one sulphur spray.

The autumn months of 1941 were almost entirely lacking in rain; the warm and dry weather favoured an epidemic outbreak of powdery mildew throughout the country, while it was less favourable for the leaf mould disease which did not spread in the coastal plain with anything like its usual rapidity. A number of cold days in early January 1942 caused extensive frost injury to tomatoes in all parts of the country. Of the experimental plots, especially the one at Nir David (Valley of Esdrælon) suffered severely as it only just began to yield, while the plots in the Jordan Valley (Degania) and the coastal plain (Mikve Israel) were by that time approaching the end of their fruiting period.

Trial No. 3, Mikve Israel.

Seedlings of the Tezier Prime variety were planted on August 28th 1941 on heavy soil. The programme of treatments was as follows:

(a) Control
(b) Sulfinette 1 1/2%
(c) Cita Lime Sulphur spray 1 1/2%
(d) Home-made Bordeaux mixture 1%
(e) Perenox 1/3%
(f) Perenox 1/3%+medium white oil 1%

Each treatment was replicated 4 times, the size of each replicate (without barrier rows) was approximately 35 sq. metres. The sprays were first applied shortly after appearance of leaf mould. The first 3 applications were made at weekly intervals, but after the appearance of leaf mould early in October the intervals were reduced to 4-5 days. A total of 19 applications was made between September 19th and December 20th 1941. The rate of spraying per dunam averaged 100 litres at the first 5 applications, 140 litres at the following 3 applications, and 175-200 litres at the remaining applications. Only in the strongly developed plants of the lime-sulphur plots the sprays were in the end applied at a rate of 250-280 litres per dunam. Fruits were picked at 4-7 days' intervals between November 23rd 1941 and February 12th 1942.

RESULTS. — The first records of powdery mildew and of leaf mould were made on September 13th and October 3rd 1941, respectively. When the incidence of diseases was estimated on November 27th the control plants were found to be affected by both powdery mildew and leaf mould on more than half the surface of the leaves of their basal part (2 marks) and on approximately half that surface in their central part (1 mark). The comparative incidence of diseases in the treatment plots is set out in table II.

The results show that both sulphur and copper sprays were very effective against leaf mould, while only the sulphur spray controlled powdery mildew satisfactorily.

All plots suffered severe frost injury on the night of January 5th 1942 and we were thus unable to estimate the incidence of diseases for a second time. It must be added that the plants sprayed with Bordeaux mixture and with Perenox were low in height, while those sprayed with Perenox and oil were taller and more strongly developed, and the plots sprayed with Sulfinette or Cita Lime Sulphur were very vigorous in their growth.

TABLE II.

Effect of Treatments on the Incidence of Powdery Mildew and Leaf Mould and on the Size and Distribution of Yields

Treatment	Block	Comparative incidence (average of 2 plots) of		Yield in kg.			% Yield (Control = 100%)	% Yield at the end of the picking season 6.1.—12.2.1942 (total yield per treatment = 100 %)	
		powdery mildew	leaf mould	per plot	mean	per dunam		per plot	mean
	A			7.0				7.8	
Control	B	100	100	17.2	15.7	480	100	7.9	19.3
	C			19.1				43.5	
	D			18.3				18.1	
	A			20.7				61.3	
Sulfinette	B	3	18	46.0	37.9	1170	241	43.6	61.0
1.5%	C			56.4				64.7	
	D			28.4				74.2	
	A			19.3				45.9	
Cita	B			30.5	32.3	990	206	56.9	59.0
Lime-	C	3	3	49.5				67.9	
Sulphur 1.5%	D			29.9				65.3	
	A			8.5				23.8	
Bordeaux	B	50	0	19.2	23.6	730	150	15.6	29.1
Mixture	C			34.6				41.1	
1%	D			32.0				36.2	
	A			9.7				13.6	
Perenox	B	30	0	18.1	20.0	615	127	12.1	28.6
1/3%	C			30.2				47.2	
	D			22.1				41.5	
	A			17.8				62.1	
Perenox	B	30	0	28.0	29.9	920	190	44.4	61.1
1/3%	C			41.3				70.4	
+oil 1%	D			32.4				67.7	
Significant difference:					8.50	260	54.1		11.33

The effect of treatments on size and distribution of yields is apparent from table II. Sulfinette, Cita Lime Sulphur, and Perenox + oil increased the yield significantly as compared with the control and with the treatment by Perenox alone. Sulfinette and Cita Lime Sulphur outyielded the Bordeaux mixture plots significantly. Bordeaux mixture and Perenox did not significantly increase the level of yields above that of the control. The table indicates that under all treatments yields were particularly low in block A which was situated at the end of the irrigation channels and suffered from lack of water. Special interest attaches to the fact that in this block spraying with Perenox and Bordeaux mixture, notwithstanding their fungicidal efficacy, failed altogether to increase the yield.

As regards the effects of treatments on the distribution of yield, Bordeaux mixture and Perenox did not significantly increase the percentage of fruit picked at the end of the season; Perenox + oil, Sulfinette, and Cita Lime Sulphur on the other hand, increased this percentage very greatly and thus prolonged the picking season considerably.

The above yield increases due to spraying were obtained in a year in which frost injury terminated the yield prematurely. At that time the control plots had dried up almost completely and had ceased to yield anyway, but the treatment plots were still green and in full bearing. If it had not been for the frost, the yield increase resulting from the treatments would therefore have been still larger.

Trial No. 4, Degania (Jordan Valley).

Seedlings of the Marmande variety were planted out on August 25th 1941 on medium, calcareous soil typical of the Jordan Valley. The programme of treatments was as follows:

(a) Control.
(b) Sulfinette 1.5%
(c) Home-made Bordeaux mixture 1%
(d) Perenox 1/3%
(e) Perenox 1/3% + medium oil 1%
(f) Cuprogreen Concentrated 1/3%

(b) - (f) applied at 4 days' intervals

(g) Perenox 1/3% + medium oil 1%, applied at 8 days' intervals
(h) Perenox 1/3% + medium oil 1%, applied at 16 days' intervals.

Each treatment was replicated 4 times, the size of each replicate being approximately 65 square metres. The first application of sprays was made on September 30th 1941, before the appearance of any symptoms of disease. Further applications of treatments (b) - (f) were made at first at 8 days' intervals, on October 8th and 16th, and after appearance of symptoms of powdery mildew at shorter intervals on October 22nd and 27th, and on November 2nd, 6th, and 13th. These plots therefore received a total of 8 applications up to the middle of November. Treatment (g) was applied at every second of the above applications, and treatment (h) at every fourth application, so that the latter plots were sprayed only on September 30th and October 27th.- On November 20th the entire experimental field was sprayed with Sulfinette 1.5%, and on December 5th a further application of this spray was made to the plots under treatment (b). The plots neighbouring the experimental field, which were included in our estimations for the sake of comparison, were sprayed with Sulfinette 1.5% on October 16th, dusted with sulphur on October 23rd, sprayed again on November 6th and 20th and finally dusted on November 26th 1941, the number of treatments totalling 5.- The rate of spraying on the experimental plots averaged 100 litres per dunam at the first and 150 litres at the last applications.- The fruits were picked at 4-5 days' intervals in 8 pickings at longer intervals up to January 26th 1942. At the final picking the plots treated with Sulfinette were still bearing a considerable amount of green fruit, while the remaining plots had altogether ceased to yield.

RESULTS. — Powdery mildew was first recorded during the first week of October 1941. The dates and results of our estimation of the incidence of mildew in the trial and on the neighbouring plots are set out in table III.

TABLE III.

Effect of Treatments on the Incidence of Powdery Mildew and on the Size and Distribution of Yields

Treatment	Block	Comparative incidence of powdery mildew (average of 4 plots) 1941 5.11	15.11	19.11	15.12	Yield in kg. per plot	mean	per dunam	% Yield (Control = 100%)	% Yield at the end of the picking season 1.1—26.1.1942 (total yield per treatment=100%) per plot	mean
Control	A	6	14	39	100	300.2	300.8	2830	100	16.2	18.8
	B					295.9				17.8	
	C					292.7				15.7	
	D					314.3				25.5	
Sulfinette 1.5%	A	0	2	8	22	416.3	451.9	4250	150	46.9	41.2
	B					455.3				39.3	
	C					487.9				43.8	
	D					448.1				34.6	
Bordeaux Mixture 1%	A	0.6	14	33	95	304.1	327.8	3080	109	14.5	19.3
	B					332.2				16.2	
	C					333.3				20.3	
	D					341.7				26.2	
Perenox 1/3%	A	4	21	33	95	343.1	310.6	2920	103	21.6	23.1
	B					301.2				19.4	
	C					278.4				12.0	
	D					319.6				39.4	
Cupro-green Concentrated 1/3%	A	3	17	33	95	318.5	346.9	3260	115	15.1	21.0
	B					338.0				20.7	
	C					361.3				18.0	
	D					370.0				30.0	
Perenox 1/3% +oil 1%	A	0.6	10	31	83	290.9	334.6	3150	111	27.1	26.6
	B					317.4				20.2	
	C					354.5				29.3	
	D					375.6				29.9	
Perenox 1/3% + oil 1% every 2nd application	A	2	17	31	83	285.7	334.2	3140	111	11.7	26.0
	B					329.9				33.8	
	C					384.8				24.1	
	D					336.4				34.2	
Perepox 1/3% + oil 1% every 4th application	A	6	17	35	89	308.6	345.1	3240	115	13.0	25.3
	B					300.7				26.4	
	C					359.6				32.3	
	D					411.3				29.4	
Neighbouring plots		-	3	9	22						
Significant difference:							39.2	370	13.0		5.5

The table clearly indicates that of all the experimental treatments only Sulfinette - and in the neighbouring plots Sulfinette and sulphur dusting - effectively controlled powdery mildew. All the copper sprays were ineffective. Infection with powdery mildew was slightly less on the plots sprayed with Perenox and oil than on those sprayed with Perenox alone.

All the plots suffered severe frost injury on the night of January 5th, 1942.

The effect of treatments on the size and distribution of yields is apparent from table III. These figures show that (a) Sulfinette significantly outyielded the control and all the other treatments; (b) treatment with Cuprogreen Concentrated and with Perenox + oil (every fourth application) significantly outyielded the control, but not the other copper treatment; (c) the addition of oil to Perenox resulted in a slight, but not significant, increase in yield as compared with the plots sprayed with Bordeaux mixture or with Perenox alone.

The percentage of fruit at the end of the picking season was very much higher on the Sulfinette than on all the other plots, and this treatments thus prolonged the picking period considerably. Among the remaining plots, those treated with Perenox + oil significantly exceeded the control and those sprayed with Bordeaux mixture or Cuprogreen Concentrated in the percentage of their late fruit.

Attention must be drawn to the fact that the actual effect of Sulfinette on the yield was even more pronounced than would appear from table III, for the following reasons: (1) At the end of the picking period, when the leaves on the control and copper treated plots dried up, the fruits picked from these plots were mostly soft and unfit for marketing, while the Sulfinette plots were still well-covered with leaves and continued to yield firm, marketable fruit; as we were unable to grade the fruits from each plot separately, this important fact failed to find numerical expression in our results. (2) When frost injured the field, the Sulfinette plots were still in full bearing, while the remaining plots approached the end of their yield. (3) In spite of this frost injury the Sulfinette plots did not entirely cease to yield, but picking was terminated for technical reasons.

Trial No. 5, Nir David (Eastern Valley of Esdrælon).

Tomatoes of the Marmande variety were planted out on September 17th 1941 on the highly calcareous soil typical of the region. The spraying programme was as follows:

(a) Control
(b) Sulfinette 1.5%
(c) Home-made Bordeaux mixture 1%
(d) Flordo Spray
(e) Perenox 1/3%
(f) Cuprogreen Concentrated 1/3%
(g) Perenox 1/3% + medium white oil 1%

applied at 8 days' intervals

(h) Perenox 1/3% + medium white oil 1%, applied at 16 days' intervals.

Each treatment was replicated four times, each replicate covering 60 square metres. Spraying commenced on October 14th, before the appearance of disease symptoms, and a further 7 applications were then made at 8-9 days intervals up to December 12th 1941. A prolonged spell of rain then necessitated an interval of 25 days until the ninth application could be made on January 6th 1942. The rate of spraying rose from 110 litres per dunam at the first to 200 litres at the last 5 applications. Treatment (h) was applied on October 14th and thereafter at every second application of the remaining treatments.- The fruits were picked at 2-4 days intervals between December 31st 1941 and February 27th 1942.

RESULTS. — The first symptoms of powdery mildew were recorded on November 5th 1941. The disease spread only slowly, and on December 9th infection on the control plots was still very slight (0.1 marks). The plots treated with copper sprays were also very slightly infected, while the Sulfinette plots were entirely free from mildew.

On the night of January 5th 1942 the temperature fell for a few hours to the low level of 0 to -1°C. The experimental fields suffered severe injury, the extent of which was seen to vary somewhat under the different treatments, we therefore estimated the extent of frost injury to the leaves by the scale of marks outlined on p. 120 and counted the number of frost-injured fruits on each plot. The results of these estimations and counts have been included in table IV, which also indicates the effect of treatments on the size and distribution of yield.

On the date of our estimation (January 16th) frost injury to the leaves was found to have been markedly reduced by the Perenox and oil spray, but was not affected by any of the other treatments. However, when the plots were again inspected about a fortnight later, the above differences were no longer apparent.

The number of fruits injured by frost was significantly reduced by all the treatments. The plots receiving copper treatments did not differ significantly from one another in the amount of frosted fruit, but all, except Flordo Spray, yielded significantly less such fruit than the Sulfinette plots.

TABLE IV.

Effect of Treatments on the Extent of Frost-Injury to Leaves and Fruits and on the Size and Distribution of Yields

Treatment	Block	Frost Injury to leaves (disease rating) per plot	Frost Injury to leaves (disease rating) mean	No. of fruits injured by frost per plot	No. of fruits injured by frost mean	% (Control=100%)	Yield in kg. per plot	Yield in kg. mean	Yield in kg. per dunam	% Yield (Control=100%)	% Yield at the end of the picking season 7.2–27.2.1941 (total yield per treatment=100%) per plot	% Yield at the end of the picking season 7.2–27.2.1941 (total yield per treatment=100%) mean
Control	A	3		127			227				5 8	
	B	3	3.0	313	252.3	100	217	214.5	3430	100	8.7	10.6
	C	3		206			209				17 5	
	D	3		363			205				10.4	
Sulfinette 1.5%	A	4		201			218				12.4	
	B	3	3.5	176	191.8	76	236	201.5	3220	94	9,6	10.0
	C	3		188			185				8.9	
	D	4		202			167				9.5	
Bordeaux mixture 1%	A	3		77			230				8.5	
	B	2	3.0	124	110.8	44	131	189.5	3030	88	7.0	11 5
	C	3		98			188				15.6	
	D	4		144			209				14.8	
Flordo Spray	A	4		104			247				13.1	
	B	4	3.0	173	151.8	60	199	200.8	3210	94	11.0	10.6
	C	2		126			179				8.2	
	D	2		204			182				10.0	
Perenox 1/3%	A	2		75			230				15.1	
	B	3	2.3	139	116.3	46	138	174.0	2780	81	15.0	
	C	1		107			142				8.8	
	D	3		144			186				14.8	
Cupro-green Concentrat-ed 1/3%	A	4		92			238				14.9	
	B	3	2.7	175	127.5	51	207	219.2	3510	102	8.4	8.8
	C	1		131			223				3.0	
	D	3		112			209				8.9	
Perenox 1/3% + oil 1%	A	1		79			203				17.2	
	B	1	1.4	127	112.8	45	164	203.8	3260	95	15.4	13.7
	C	1.5		103			235				12.0	
	D	2		142			213				10.0	
Perenox 1/3% + oil 1%, every 2nd application	A	2		109			221				5.7	
	B	2	1.9	153	124.8	49	176	204.5	3270	95	4.4	8.2
	C	1.5		102			226				8.5	
	D	2		135			195				14.3	
Significant difference:					51.5	20.4		38.3	610	17.7		5.65

None of the treatments increased the yield, and only Perenox at 1/3% strength reduced the yield significantly. The difference in yield between the plots sprayed with Perenox and oil and those sprayed with Perenox alone was not significant, but the addition of oil prevented the significant reduction of yield obtained with Perenox alone.

The treatments did not significantly affect the percentage of fruit picked at the end of the season. This may be assumed to be due to the fact that the yields of all plots were curtailed by frost injury to the leaves. As stated above, most treatments did not reduce this injury in any way, and even the reduction observed on the Perenox and oil plots seems to have been too slight to find its expression in an increase of total yields or of the percentage of late yield.

IV. DISCUSSION

A. Fungicidal action of sprays against powdery mildew and leaf mould

1. Powdery Mildew (*Leveillula* (*Oidiopsis*) *taurica*).

Literature references to the appearance of this disease on tomatoes are rare and no control experiments whatever appear to be on record.

Our 1941 trials in the coastal plain and in the Jordan Valley proved lime-sulphur sprays to be a most effective means of combating powdery mildew. At 1.5% strength both Sulfinette and Cita Lime-Sulphur reduced the incidence of disease by more than 80%. as compared with the control, and protected the leaves from mildew attack throughout the growing season. In the trial in the Jordan Valley, where powdery mildew alone attacked the crop, the effect of these sprays was particularly pronounced, as the plants so treated remained vigorous and green for at least a month longer after the control plants had dried up entirely.

Copper sprays, such as home-made Bordeaux mixture at 1%, Perenox and Cuprogreen Concentrated at 1/3% strength, or Perenox 1/3% with the addition of 1% oil, were only slightly effective against powdery mildew and were much inferior to the lime-sulphur sprays. In the Jordan Valley in 1941, Perenox with oil was somewhat more effective than without oil.

2. Leaf Mould (*Cladosporium fulvum*).

Numerous trials for the control of tomato leaf mould have been carried out with all the common, and some of the less common, fungicides wherever the disease is troublesome. In the following section we shall discuss the results reported from abroad in relation to those of our trials.

(a) HOME-MADE BORDEAUX MIXTURE. — Effective leaf mould control by means of Bordeaux mixture, ranging from 0.5% to 2% in strength, has been reported from Germany (4,26) as early as 1926. In our trials this spray was tested against leaf mould only in the 1939 and 1941 experiments in the coastal plain. In 1939 five applications made at 3 weeks' intervals entirely failed to control the disease, while in 1941 frequent applications of 1% Bordeaux mixture at 5 days' intervals suppressed leaf mould almost entirely and thus confirmed the results obtained by the German writers.

(b) "INSOLUBLE" COPPER SPRAYS. — These materials, with or without the addition of oil, have so far mainly been used for leaf mould control in England and New Zealand. In England READ (14, 15) controlled the disease by spraying with copper oxychloride and copper-zinc silicates, and obtained particularly favourable results with cuprous oxide with the addition of oil. He used the cuprous oxide at approximately 1/6% strength which corresponds in copper content to the proprietary cuprous oxide spray Perenox used at 1/5-1/4%. CURTIS (3) in New Zealand found cuprous oxide effective at the even lower concentration of 1/9%, corresponding to about 1/6% Perenox, to which he added cotton-seed oil.

In our trials in the coastal plain Perenox was used at 1/2% in 1939 and 1940 (in 1940 with the addition of 1% Alboleum oil) and at 1/3% in 1941 (with and without oil). In 1939 this spray, like Bordeaux mixture, failed to control leaf mould because applications were not made with sufficient frequency. But in 1940 and 1941 frequent applications proved Perenox to be a very effective means of combating leaf mould. As apparent from the 1941 trial, a 1/3% concentration of this spray was quite strong enough, and in view of the reference cited above even weaker concentration may suffice to control the disease. The addition of oil did not enhance the fungicidal efficacy of Perenox in this case.

(c) SULPHUR SPRAYS. — Leaf mould control by sulphur sprays has been recommended from many countries. The barium polysulphide spray Solbar was widely advocated for this purpose in Germany (6, 7, 13) and lime-sulphur sprays of 1/2% strength were successfully employed in England and elsewhere. SCHOEVERS (18) found 1.5% lime-sulphur sprays to be more effective than Bordeaux mixture. SMALL (21) stated that Solbar and sodium polysulphide spray failed to control leaf mould, while colloidal sulphur spray succeeded in doing so.

Our trials included the sodium pentasulphide spray Sulfocide (1939 and 1940), and the lime-sulphur sprays Cita Lime-Sulphur (1940 and 1941) and Sulfinette (1941). In 1939, when applied at long intervals, Sulfocide was as ineffective as the copper sprays; but in 1940 frequent spraying with this or Cita Lime-Sulphur spray gave satisfactory control of leaf mould. This result with Sulfocide contradicts SMALL's findings as to the inefficacy of sodium polysulphides. In 1941 both Cita Lime-Sulphur and Sulfinette again confirmed the efficacy of lime-sulphur sprays against leaf mould, but were slightly less effective than Bordeaux mixture or Perenox.

(d) SHIRLAN AG. — The use for leaf mould control of salicylanilide and of the Shirlan pastes based on this compound have been advocated by SMALL (21) and other English and New Zealand writers (3, 11) ever since 1931, the concentrations of Shirlan recommended for this purpose ranging from 0.08 to 0.75%. We only

tested this material in the 1940 trial at 0.5% strength and found it to be as effective against leaf mould as Cita Lime Sulphur and Perenox.

The above results indicate that copper as well as sulphur sprays may be employed for the control of leaf mould. This fact is of great practical importance as it permits the combination of leaf mould control on the one hand with control measures against diseases amenable only to sulphur treatments (powdery mildew), and on the other hand with measures for the prevention of diseases sensitive to copper, such as *Altarnaria* or *Phytophthora* blight or *Septoria* leaf spot.

B. Effect of treatments on frost injury

The results of our trial at Nir David have shown that neither Sulfinette nor the various copper treatments affected the extent of frost injury to tomato leaves. But the addition of 1% white oil to the Perenox spray resulted in a definite, though only temporary, reduction of such injury. As far as we are aware, no mention of the effect of oil sprays on frost injury has so far been made in literature.

In the same trial all the sprays markedly reduced the incidence of frost injury on the fruits. Although the differences in the number of frosted fruits under the various treatments were in most cases not significant, it is interesting to note that the number of affected fruits appeared to bear an inverse relationship to the copper content of the sprays. In the first place, all copper treated plots yielded fewer frosted fruits than the Sulfinette plots. Secondly, among the plots treated with copper sprays, the highest number of fruits injured by frost was recorded on the plots sprayed with Flordo Spray, which contains only 0.06% metallic copper. Cuprogreen Concentrated and Perenox at 1/3% strength both contain about 0.17% copper, but numerous experiments have shown that Perenox possesses copper in a more active form than Cuprogreen (16). The number of frosted fruits on the Cuprogreen plots was accordingly a little higher than that on the Perenox plots. Lastly, Bordeaux mixture which at 1% strength has a copper content of 0.25%, yielded the lowest number of fruits injured by frost.

Literature references to the effect of copper on frost injury are rare, and only SZIRMAI (22), working with wheat, has recorded some such effect.

C. Effect of treatments on the size of yields and on the duration of the period of fruiting

1. Size of Yield.

The effect of treatments on the size of yield is the result of their fungicidal action on the one, and of their phytocidal action on the other hand. The fungicidal effect can be directly determined by estimation of the leaf area affected by disease, and the previous sections

have described the results of such estimations. The phytocidal effect, however, cannot be estimated directly except in extreme cases. But every treatment effect on the plants is bound to be reflected in the size of the final yield and careful interpretation of the latter enables us to draw conclusions regarding the phytocidal action of the materials tested. We shall now deal with this aspect of our results and compare our conclusions with the views expressed in literature.

(a) COPPER SPRAYS. — In the three 1941 trials we used Bordeaux mixture at 1% strength, Perenox and Cuprogreen Concentrated at 1/3%, and Flordo spray.- At Mikve Israel (coastal plain), where the spray load was extremely heavy (19 applications), Bordeaux mixture and Perenox proved very effective against leaf mould, but failed to increase yields significantly. This fact — especially if considered in relation to the higher yields obtained by the addition of oil to the Perenox spray (cf. below) — indicates that both sprays were harmful to the plants at these high spray loads.- At Degania (Jordan Valley) and Nir David (Valley of Esdrælon) the number of applications was only eight. At Degania, the limiting factor was powdery mildew, which proved amenable to sulphur treatment only. Under these conditions Perenox and Bordeaux mixture increased the yield by only 3-9%, while Cuprogreen Concentrated increased it significantly by 15%. At Nir David no disease attack developed in the trial and all copper treatments were equally ineffective against frost injury to the leaves. Here again Bordeaux mixture and Perenox reduced the yield by 12-19%, while Flordo spray and Cuprogreen Concentrated equalled the control in yield. These results warrant the conclusion that even with lighter spray loads Bordeaux mixture at 1% and Perenox at 1/3% strength are apt to affect the tomato plants adversely and, in the absence of diseases, may reduce the yield. These sprays should therefore in future be tested at lower concentrations.

The literature on the effect of copper sprays on copper sensitive plants was recently reviewed by us in our paper on cucumber disease control (16). Bordeaux injury to tomatoes has been reported from many countries (2, 9, 10, 12, 20, 23, 24) and many authors (2, 12) emphasize that plants are especially liable to it when still young and when growing under drought conditions. HORSFALL and his collaborators (9, 10) have pointed out that the spray load may be decisive for the extent of leaf injury. In the light of these researches the harmful effect of Bordeaux mixture in our 1941 trial at Mikve Israel is readily explained, as spraying was then commenced at a very early stage of growth and the spray load was very heavy. The effect of drought in accentuating Bordeaux injury was also confirmed in this trial; as apparent from table II, spraying with Bordeaux mixture entirely failed to increase the yield in block A, which suffered from inadequate irrigation, while in the blocks with normal irrigation yields were increased by about 50%.

WILSON and RUNNELS (24) found "insoluble" copper sprays, such as copper oxychloride and cuprous oxide, to be much less injurious to tomatoes than Bordeaux mixture. HORSFALL and HAMILTON (8), however, hold that cuprous oxide does not differ from Bordeaux mixture in its phytocidal effect. In our trials Cuprogreen Concentrated, which is based on copper oxychloride, proved quite innocuous to tomatoes under conditions in which Bordeaux mixture was injurious. This result confirms WILSON and RUNNELS' findings with regard to copper oxychloride. But our results appear to disagree with these writers where cuprous oxide is concerned: Perenox, which is based on this compound, at 1/3% strength did not prove superior to 1% Bordeaux mixture in its phytocidal action, but this might possibly be due to the ingredients other than cuprous oxide incorporated in Perenox.

(b) THE ADDITION OF OIL TO PERENOX. — The 1941 trial at Mikve Israel showed that the addition of 1% medium white oil to Perenox at 1/3% strength does not enhance the efficacy of the latter against leaf mould. In this trial, where the number of applications reached 19, the plots sprayed with Perenox and oil yielded 60% more than those sprayed with Perenox alone. At Degania the addition of oil to Perenox slightly increased the yield. At Nir David the addition of oil prevented the significant reduction of yield caused by the Perenox spray alone. All these results demonstrate that the addition of 1% medium oil to a 1/3% Perenox spray is liable to prevent spray injury and may thus increase the yield substantially.

In the latter two trials a comparison was made of the effect of 8 and of 4 applications of the Perenox and oil spray (at Degania also of 2 applications). If this spray had affected the tomato plants in any way, the considerable difference in spray load between the plots receiving different numbers of applications should have found its expression in a corresponding difference in yield. The absence of any such difference constitutes further evidence for the complete phytocidal safety of the combined Perenox and oil spray.

In the 1940 trial 1% Alboleum oil was added to a 0.5% solution of Perenox. Altough this concentration of Perenox may definitely be thought to affect the plants adversely, and in spite of the very high spray load (16 applications) and the tender stage of growth at which spraying commenced, the Perenox and Alboleum spray increased yields by 35%. While no direct comparison between the effect of Perenox with and without oil was made in this trial, this result, especially if considered in relation to the yield obtained with Cita Lime-Sulphur (cf. below) in the same trial, supports our conclusion that the addition of oil to Perenox prevents spraying injury to tomatoes.

Various American authors have stated that the addition of cotton-seed or mineral oil to Bordeaux mixture (2, 10, 23, 24) or

to cuprous oxide spray (9, 28) prevents injury to tomatoes. HORSFALL and HARRISON (10) have shown that the rate of transpiration of young tomato plants sprayed with cotton-seed oil with or without the addition of cuprous oxide was lower than that of unsprayed control plants. These writers as well as WILSON and RUNNELS (23) hold that the oil prevents the excessive water loss that may be brought about by spraying with Bordeaux mixture or cuprous oxide alone, and thus protects the plants from injury. These findings explain why the action of the oil is particularly evident under drought conditions, a phenomenon which has variously been mentioned in literature (2, 23) and was confirmed in our cucumber spraying trials (16). Further evidence on this point has been furnished by our 1941 tomato trial at Mikve Israel, where the plots sprayed with Perenox and oil outyielded those sprayed with Perenox alone by 44% in the blocks which received adequate irrigation, but by 85% in one block which suffered from lack of water (table II).

(c) SULPHUR SPRAYS. — In the 1940 trial the sodium pentasulphide spray Sulfocide was effective against leaf mould, but its effect on the yield was not clear, and we cannot therefore assess its phytocidal action. Cita Lime-Sulphur, in both 1940 and 1941, and Sulfinette, in two of the 1941 trials effectively controlled powdery mildew and leaf mould and greatly increased the yield. These results may be taken to indicate that at the concentration of 1.5% employed in the trials, these two sprays do not affect the tomato plants. This was confirmed at Nir David, where Sulfinette — in the absence of diseases — neither increased nor diminished the yield. We are not aware of any literature references to lime-sulphur injury to tomatoes.

(d) SHIRLAN AG. — At 0.5% strength Shirlan AG was effective against leaf mould in the 1940 trial. But, as in the case of Sulfocide, the yields obtained from this treatment were not conclusive, and we could not therefore assess its phytocidal properties. Considerable increases of yield by spraying with Shirlan AG have been reported especially from New Zealand (3, 11).

2. Duration of the picking period.

At Mikve Israel in 1940 and 1941 and at Degania in 1941, some of the treatments increased not only the total yield, but even much more the percentage of fruits picked at the end of the season. Thus Cita Lime-Sulphur, Perenox and Alboleum, and Shirlan, in the 1940 trial, exceeded the total yield of the control by 13-35%, but increased the percentage of late fruit from 7.6% in the control to 25-30%, i. e. 3-4 times. At Mikve Israel in 1941 the lime-sulphur sprays and Perenox and oil spray increased total yields by 90-140%, but raised the percentage of late fruit more than 3 times, from 19% in the control to about 60%. At Degania, Sulfinette outyielded the control by 50%, but more than doubled the percentage of fruit picked at the end of the season (from 18.8% to 41.2%). In the same trial

treatment with Perenox and oil significantly increased this percentage, though it resulted in only a slight increase of total yields.

The above results demonstrate in the first place that the increase in percentage of late fruit is generally largest where total yields are also greatly increased, such as in the case of the lime-sulphur sprays in the 1941 trials at Mikve Israel and Degania. But we further observe that the size of the increase of total yield does not always correspond with that of the percentage of late fruit. Thus Sulfinette, in the 1941 trial at Mikve Israel, outyielded Perenox and oil by about 30%, but the treatments equalled one another in the percentage of fruit picked at the end of the season. At Degania, treatments with Cuprogreen Concentrated, Bordeaux mixture, and Perenox and oil, resulted in approximately equal total yields, but the latter treatment yielded a significantly higher percentage of late fruit than the Cuprogreen or Bordeaux sprays. The results of both these trials appear to indicate, that the addition of white oil has a special effect on the distribution of yields, tends to increase the percentage of late fruit, and thus extends the picking period. A similar effect of the oil was noted in our cucumber spraying trials (16, and recent results not yet published). We were unable to find any literature references to the effect of spray-oils on the duration of the picking period.

The opinion has variously been expressed abroad (20, 25) that spraying with Bordeaux mixture delays the ripening of tomato fruits. No confirmation of this opinion has been forthcoming in our trials, as the percentage of late fruit on the Bordeaux plots failed to exceed that of the control plots to any marked extent.

D. *Spraying intervals and profitability of treatments*

1. Spraying Intervals.

The frequency with which sprays are to be applied evidently depends on the rapidity of the development of the diseases and of the plants. Some data pertaining to this problem have so far been obtained by us in the case of powdery mildew only in the 1941 trial at Degania, and in the case of leaf moud in the 3 trials at Mikve Israel in 1939-1941.

(a) powdery mildew. — As apparent from the estimations detailed in table II, powdery mildew at Degania in autumn 1941 developed slowly from the date of its first appearance in early October until the beginning of November. In mid-November, however, its development grew so rapid that the disease spread within 4 days from the basal third of the plants to their top third (about 70 cm above soil level). — Certain conclusions regarding the frequency of treatment applications for the control of powdery mildew under these conditions may be drawn from a comparison of the experimental plot which received 10 applications of Sulfinette, and the neighbouring plots outside the experiment, which received 5 applications of Sulfinette and

sulphur. In the latter plots three treatment applications, beginning well after appearance of the disease, were made until early November, and only 2 further applications were made at comparatively long intervals during the period at which the powdery mildew reached the climax of its development. The fact that these plots were as little affected by powdery mildew as the Sulfinette plots, shows that the frequency of applications was not decisive in this case, but further study is required to determine more accurately the length of intervals most profitable to the grower. Preventive spraying is apparently not necessary for the control of the mildew, as treatments begun only after appearance of the disease succeeded in controlling it satisfactorily.

(b) LEAF MOULD. — The failure of our 1939 trial clearly demonstrated that under the conditions prevailing in the coastal plain during the autumn months, leaf mould develops so rapidly that it cannot be controlled by applications at 3 weeks' intervals. The 1940 and 1941 trials proved that under these conditions spray applications made at 4-5 days' intervals, beginning immediately after the appearance of the first symptoms of disease, give excellent control of leaf mould. No comparison of the efficacy of various spraying intervals has yet been made in our trials, but we consider it likely that no less effective control may be obtained if treatment intervals during the second half of the growing season are lengthened to some extent.

The treatment intervals recommended in literature for the control of leaf mould vary widely. Some authors (13) hold that applications at 3 weeks' intervals suffice to check the disease in the greenhouse, but such is certainly not the case under field conditions in Palestine, American writers (5, 27) have advocated weekly applications, and similar intervals may also approach the economic optimum under the climatic conditions prevailing here in the autumn and winter months. In 1927, the opinion has been voiced in Germany (26) that the choice of suitable treatment intervals is of far greater moment for the control of leaf mould than the choice of the fungicide used for the purpose. Our results fully confirm this view, as we found copper and sulphur sprays, as well as the salicylanilide spray Shirlan AG, to be effective means of controlling the disease, provided they were applied at frequent enough intervals.

2. Profitability of Treatments.

Although we are as yet unable to state accurately the number of applications necessary for the control of the diseases dealt with here, we may assume that in the course of a growing season, not curtailed by frost, at least 8 applications will be required under conditions favouring powdery mildew, but not leaf mould, and at least 12 applications where leaf mould is apt to develop strongly.

At the present level of prices each application costs about L.P. 0.500 per dunam. With the high level of yields usual in the

Jordan Valley, a 50% increase of yield, as obtained by Sulfinette spraying in the Degania trial, represents about 1.5 tons of additional yield per dunam. There can be no doubt that the extra income derived from this yield is far in excess of the L.P.4.- expenses incurred in 8 applications, and here spraying will definitely be profitable.

At Mikve Israel the attack of leaf mould on autumn grown tomatoes is usually so strong, that unsprayed plots will not yield enough to cover cultural expenses. On the other hand, the level of yields of tomatoes grown under these conditions is never more than moderately high, even if the crop is effectively protected from leaf mould, and it is questionable whether even a large percentage increase of yield will then suffice to cover the expenses of about L.P.6.- incurred in 12 applications. In this case profitability of the crop entirely depends on the level of prices. If the latter are not high enough to make a moderate-sized crop repay the usual cultural expenses as well as the spraying expenses indispensable in this case, the cultivation of tomatoes under these conditions cannot be considered remunerative.

V. SUMMARY.

(1) Spraying trials for the control of tomato diseases have been carried out for three years (1939-1941) in the central coastal plain of Palestine, and one year (1941) in the Jordan Valley and the eastern Valley of Esdrælon.

(2) In the 1940 and 1941 trials the fungicidal effect of treatments in controlling the powdery mildew (*Leveillula* (*Oidiopsis*) *taurica*) and leaf mould (*Cladosporium fulvum*) diseases has been estimated according to the leaf area affected. Similar estimations were made of the effect of treatments on leaf injury due to frost.

(3) Spraying with the lime-sulphur preparations Cita Lime-Sulphur and Sulfinette at 1.5% strength gave very effective control of powdery mildew, while copper sprays were only slightly effective.

(4) Excellent control of leaf mould was obtained by spraying with the above lime-sulphur washes, with the sodium pentasulphide spray Sulfocide and the salicylanide spray Shirlan AG, both at 0.5%, and with Bordeaux mixture at 1% and the cuprous oxide spray Perenox at 0.33 or 0.5%. The addition of 1% white oil to the Perenox spray did not affect the efficacy of the latter.

(5) None of the sulphur or copper sprays had any effect on the extent of frost injury to the leaves, but spraying with Perenox and oil effected a definite, though temporary, reduction of such injury in one of the trials.

(6) In the same trial the number of tomato fruits injured by frost was markedly reduced by Sulfinette, Bordeaux mixture, Flordo spray, Perenox with or without oil, and Cuprogreen Concentrated (based on copper exychloride). The number of frost injured fruits

appeared to bear an inverse relationship to the metallic copper content of these sprays.

(7) The control of powdery mildew by Sulfinette in the trial in the Jordan Valley increased yields by 50% (from 2830 to 4250 kg. per dunam=1000 square metres).

(8) The combined control of powdery mildew and leaf mould in the 1941 trial in the coastal plain effected a very large increase of yield by Cita Lime-Sulphur, Sulfinette, and Perenox and oil spray, while Bordeaux mixture and Perenox alone failed to increase the yield markedly.

(9) The interpretation of the fungicidal efficacy of these sprays and of their effect on yields, as evinced especially in the 1941 trials, have led to the conclusion that Bordeaux mixture at 1% and Perenox at 1/3% are apt to cause spray injury to tomatoes when applied at frequent intervals, and these sprays should in future be tested at lower concentrations. The addition of 1% white oil or Alboleum to the Perenox solution prevented such injury. Cuprogreen Concentrated, Flordo spray, and the lime-sulphur preparations were found to be innocuous to tomato plants even in the absence of diseases.

(10) The percentage of fruit picked at the end of the season was markedly increased by Cita Lime-Sulphur, Sulfinette, Perenox and oil, and Shirlan AG, and these treatments thereby prolonged the duration of the picking period. The Perenox and oil spray appeared to be particularly active in this respect.

(11) Observations made in the Jordan Valley in 1941 warrant the tentative conclusion that under the conditions prevailing there powdery mildew may well be controlled by lime-sulphur applications at approximately weekly intervals, even if spraying is delayed until after appearance of the first symptoms of mildew.

(12) The trials in the coastal plain have shown that leaf mould cannot be checked by fungicidal application at 3 weeks' intervals. Spraying at 4-5 day's intervals effectively controlled the disease, and the choice of suitable intervals is considered to be more important in leaf mould control than the choice of the fungicide employed for the purpose.

(13) Under conditions prevailing in the Jordan Valley the extra income obtained by spraying may safely be assumed to make frequent applications remunerative. In the coastal plain, where yields are often lower, the profitability of treatments — and thereby of the entire autumn crop which cannot be grown without spraying — depends on the level of market prices.

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Corrigendum: Instead of SCLEROTIUM BATATICOLA (Taub) Butler
read SCLEROTIUM BATATICOLA Taubenhaus.

SCLEROTIUM BATATICOLA (TAUB.) BUTLER ON POTATOES IN PALESTINE *)

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(With 2 Text-figures)

Sclerotium bataticola (Taub.) Butler appears in Palestine on potatoes in three different forms, viz. as charcoal rot or dry stem-end rot of tubers or as stem blight and root rot. As the damage caused has frequently been severe, a study was made of the host-parasite relationships and of the effect of environmental conditions on the development of this disease.

I. SYMPTOMS

(a) *Stem blight and root rot*

The entire plant suddenly turns yellow and subsequently withers. The cortex of the stem then sloughs off, exposing a dry, greyish xylem; the pith is partly destroyed and filled with sclerotial bodies which are at first whitish, later black in colour. Sclerotia are also found on the roots and stolons (text-fig. 1).

Text-fig. 1. Stem end and root rot of potatoes caused by *S. bataticola*. The stems, collar, roots and stolons are covered with black sclerotia.

*) Received for publication in August 1943.

(b) *Tuber rots*.

(1) *Dry stem end rot.* A black, sunken area develops around the stem end of the tuber. A cavity of about 10 mm. depth, filled with collapsed tuber tissue, black sclerotial bodies, and greyish cottony mycelium, forms beneath the epidermis of the lesion and is separated from healthy tissue by a layer of suberized cells. This type of rot generally remains dry and localized and does not involve the whole tuber. In most cases the stolon to which the tuber is attached is also affected. It appears, therefore, that this tuber rot should be considered as part of the stem blight and root rot described above.

(2) *Black or charcoal rot.* Black spots, encircling the lenticels and measuring 2-3 mm. in diameter, appear on the tubers. The lenticels remain visible as light-coloured points in the centre of the black spots. The skin itself is found to remain unaffected, and only the flesh beneath it is blackened. When the skin with its lenticels is peeled off, the spots in the flesh therefore appear uniformly black. These spots are granulate in appearance and extend into the flesh to a depth of about 2 mm. A soft rot, probably due to secondary organisms (bacteria), later develops from these spots and the tuber disintegrates entirely (text-fig. 2).

Text-fig. 2. Potato tuber affected by charcoal rot caused by *S. bataticola*. The white spots in the centre of a black ring are lenticels surrounded by masses of black sclerotia. The tuber is chiefly affected on the part that was topmost in the soil. Blackening of the tissues commences on the right.

This type of rot may develop on the tubers prior to lifting, when they are still in the soil, or during subsequent storage. Tubers affected by this rot to even a slight extent are insipid in taste and unfit for culinary or seed purposes.

II. DISTRIBUTION AND SEASONAL OCCURRENCE

The first record in Palestine of *Sclerotium bataticola* on potatoes was made in 1926, when it was found to be the cause of stem blight, and in the following year, when it caused tuber rots (13, 14, 15). Both these diseases, especially the tuber rot, have since been found in all parts of the country, in the coastal plain as well as in the mountainous regions and in the inner valleys. Tuber rot caused by *Sclerotium bataticola* has further been reported from India (5,9), from Georgia and Illinois in the United States (2,3), and from Cyprus (8, 10, 11).

In Palestine, *Sclerotium bataticola* almost exclusively attacks the potato crop sown in spring, i. e. in February and March, and symptoms of both stem blight and tuber rot appear during the last weeks of growth, in June and July. Tuber rots continue to develop during subsequent storage in non-refrigerated stores, but are almost absent from tubers kept in cold storage.

The disease was found to affect all the principal varieties of potatoes grown in Palestine, such as Up to Date and Arran Banner. Considerable differences in the severity of attack were observed among these varieties. However, the data so far available are insufficient to indicate, whether these differences were due to environmental or cultural factors or to various levels of resistance of the varieties concerned.

III. ECONOMIC IMPORTANCE

The stem blight caused by *Sclerotium bataticola* on potatoes is not of very frequent occurrence in Palestine, but whenever it appears the losses caused are severe.

The charcoal tuber rot, on the other hand, is very widespread and may cause very considerable damage. When the crop is being lifted, the proportion of affected tubers is usually found to be still low and does not exceed 5 per cent. of the yield. But the percentage of affected tubers counted at the time of lifting may be assumed to be somewhat too low, because a considerable number of infected tubers may entirely rot in the soil before they are lifted, and may thus escape our notice. If the tubers are subsequently stored under conditions favouring disease development, losses may reach 50 per cent. of the yield or even more.

IV. THE FUNGUS

Cultures from pieces of wilted stem always develop the typical sclerotial bodies of *Sclerotium bataticola* (Taub.) Butler (4, 16), generally in association with *Fusarium* and sometimes with *Colletotrichum atramentarium* (Nerk. et Br.) Taub. Isolations made at an early stage of disease development from potato tubers affected by charcoal rot yield almost pure cultures of *Sclerotium bataticola;* when made at a later stage of infection the cultures sometimes in-

cluded, besides *Sclerotium bataticola,* bacteria of the *carotovorus* group. These bacteria may be assumed to be the cause of the soft rot described above.

The size of sclerotia of *Sclerotium bataticola* isolated from potatoes measured 43 - 101μ x 50 - 130μ, the average being 60 x 80μ. The fungus therefore appears to belong to HAIGH's (6,7) "C" group of *Sclerotium bataticola.* The pycnidial stage of the fungus, *Macrophomina phaseoli* (Maubl.) Ashby (1), has, however, not yet been found here.

In cultures on 2% potato dextrose agar the optimum temperatures for the development of sclerotia ranged from 25 to 35°C, while the fungus failed to grow at 41°C and 7°C. TOMPKINS and GARDNER (17) found the average optimum temperature of all the strains they examined to be 31°C, and this is in line with our findings.

Sections through the small black lesions described above as representing the usual symptom of disease on potato tubers showed that the black and granulate appearance of these lesions is due to the fact that they are filled with black sclerotia; only traces of mycelium could be detected there. The surrounding non-blackened tuber tissue contained neither mycelium nor sclerotia. As mentioned above, the lesions originally produced are later often surrounded by black, watery tissue which is likewise usually free from mycelium and frequently contains bacteria. The latter may therefore be chiefly responsible for the final softening of wider zones of tuber tissue.

In inoculation tests *Sclerotium bataticola* failed to develop on potato tubers, whether wounded or not, at temperatures of 20, 25, 30, 35, and 40°C. At 25 and 30°C, mycelium and sclerotia were observed to develop on the surface of the tuber, but did not penetrate into the flesh. In another test potato tubers were first kept for two hours at high humidity at a temperature of 55°C and were then inoculated and kept at 30°C; the disease then developed in the majority of cases with all the typical symptoms. *Sclerotium bataticola* could be re-isolated from these tubers, but always in association with bacteria. These preliminary results tend to show that the disease develops only in tubers previously subjected to and injured by high temperatures, but the point requires confirmation.

V. RELATION OF ENVIRONMENT TO DISEASE DEVELOPMENT IN THE FIELD

The experiments outlined above indicate that the tuber rot develops mainly at high temperatures approximating 30 to 35°C. Temperatures exceeding 35°C are not favourable for the development of the causal organism, but injure the tubers and render them susceptible to subsequent disease attack, when conditions are favourable.

Extremely high soil temperatures commonly occur in Palestine during the months of June, July, and August, especially in light, sandy soils. Temperature records taken by PERLBERGER (12) at Re-

hovot in 1936 and 1937 showed that maximum temperatures of 58°C may obtain in the upper 5 cm. of non-irrigated light soil. Maximum temperatures of 40°C were recorded in June and July in the corresponding layer of irrigated soil, where a maximum of 30 to 35°C was reached at a depth of 10-15 cm. However, when irrigation of this soil was interrupted for only three days, the temperature in the topmost layer rose above 50°C. Field observations concerning the incidence of *Sclerotium bataticola* are in accordance with these data. Tubers lifted early in April or May are rarely attacked by the rot, whereas those lifted late in the second half of June or in July often suffer severely, especially if they are stored at outdoor temperatures. The tuber rot fails to develop at low storage temperatures (below 8°C). Moreover, the disease is most prevalent where irrigation intervals during the summer months are long or where irrigation is discontinued more than a week before lifting, a common practice of many local farmers. The percentage of diseased tubers was further observed to be higher in the hotter top layer than in the cooler lower layers of soil. This may sometimes be demonstrated on single big tubers, the lower portion of which still remains free from disease while the upper portion is infected (text-fig. 2).

VI. CONTROL MEASURES

Since the fungus causing the disease appears to penetrate the tubers from the soil, disinfection of seed tubers is of no use. Control measures must primarily consist in the avoidance of high soil temperatures during the growth of the spring crop by means of (a) the use of early varieties, (b) early sowing and lifting, (c) choice of suitable and not excessively light soil, and (d) irrigation at frequent intervals until shortly before the crop is lifted.

When lifted, the tubers should at once be removed to cool and shady sheds, where all tubers affected by disease, wounds, or heat injury, should be discarded. The crop should then be stored either in cold storage or in as cool and dry a store as can be found. The danger of tubers being infected by contact may be lessened by storing the crop in dry sand.

VII. SUMMARY

(1) The occurrence in Palestine is recorded and the symptoms are described of a stem blight and root rot of potatoes and of two types of tuber rots, viz. dry stem-end rot and charcoal rot, all caused by the fungus *Sclerotium bataticola* (Taub.) Butler.

(2) The diseases, especially the tuber rots, are stated to be widespread in Palestine, but the attack is limited to potato crops grown in late spring. All currently grown varieties are affected.

(3) The economic importance of stem blight is slight, but that of tuber rot is very considerable, as losses up to 50% may be sustained in storage.

(4) In culture, the optimum temperature for development of sclerotia ranged from 25 to 35°C. In inoculation tests the fungus failed to infect tubers, even when they were wounded, at 25 - 40°C. But when the tubers were first heated for 2 hours at 55°C, subsequent inoculations succeeded.

(5) High soil temperatures are concluded to predispose potato tubers to attack by *S. bataticola,* and measures are indicated to avoid such temperatures in the field. Low temperatures prevent rapid spreading of the disease in storage.

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TRIALS FOR THE CONTROL OF POWDERY MILDEW ON POTATOES

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I. INTRODUCTION

The powdery mildew disease of potatoes (*Oidium sp.*) first occurred in Palestine in epidemic form in autumn 1941, and again in spring 1942 and in spring and autumn 1943. The disease has been widespread especially in the northern districts of the country, in the Galilee and in the Jordan Valley and the Valley of Esdraelon, but was rarely found in southern Palestine.

Symptoms of powdery mildew usually first appear on the haulms, leaf stalks and leaf veins, and consist of brown lesions of various size on which minute dark brown stripes can be discerned. As the disease develops, a whitish covering appears on these lesions and on both leaf surfaces.

Of the four trials described in this paper, the two carried out in spring 1942 were originally designed to control in the main *Alternaria* and *Phytophthora* blights. These trials therefore mostly comprised copper treatments and only one sulphur treatment. But the dry weather prevailing in spring 1942 did not permit the development of potato blights, and the crops were attacked by powdery mildew only. The trials thus served to compare the efficacy of copper and sulphur treatments in the control of this disease. The later two experiments, carried out in spring and autumn 1943, were expressly designed for the control of powdery mildew by various sulphur dusts and sprays.

The authors are indebted to Dr. I. Reichert and Dr. F. Littauer, of the Division of Plant Pathology, for their helpful advice on the planning and execution of these trials. Our thanks are further due to the members of the settlements of Kinneret, Tel Josef, and Kfar Gil'adi, without whose active help and interest this work could not have been carried out.

II. MATERIALS AND METHODS

The materials examined in these trials may be described as follows:

1. *Bordeaux mixture* 1%, prepared from 1 kg. copper sulphate crystals and 660 grammes hydrated lime powder in 100 litres water.

2. *Perenox,* a proprietary spray material based on cuprous oxide, containing not less than 50% metallic copper and about 8% chlorine, with a slightly acid reaction. The material is produced by Messrs. Imperial Chemical Industries, England.
3. *Cuprogreen Concentrated,* a proprietary spray based on copper oxychloride, containing approximately 50% metallic copper, and neutral in reaction. A product of Belgium.
4. *White oil* (light medium), stated to contain 80% oil, 50-64% distilling over at 636° Fahrenheit, and with 92% unsulphonated residue, produced by Messrs. Shell Co., England.
5. *Sulfinette,* a lime-sulphur spray of 33° Baumé, produced by Messrs. Imperial Chemical Industries, England.
6. *Cita Lime-Sulphur Spray,* a lime-sulphur spray of 30-32° Baumé produced by Messrs. Chemical Industries, Tel Aviv, Palestine.
7. *Spersul,* a dispersible sulphur preparation for spraying, stated to contain 73% of sulphur and to be compatible with Perenox. Produced by Messrs. Imperial Chemical Industries, England.
8. *Gaza sulphur Extra Fine grade,* a dust stated to contain 95% sulphur. 90% of the dust is stated to pass sieve No. 200, and 75% to pass sieve No. 300. Produced in Palestine.

All trials were carried out on the Up to Date variety. Sowing distances were 25-30 cm. in the rows and 70-80 cm. between the rows. In trial No 1 irrigation was by furrows and sprays were applied by means of a knapsack machine working at 4-10 atmospheres' pressure. In trials Nos. 2-4 irrigation was by overhead pipes and the sprays were applied by motor sprayers working at 20-25 atmospheres' pressure.

The yield was graded as follows: Grade A — tubers exceeding 100 grammes in weight. Grade B — tubers weighing 60-100 grammes. Grade C — tubers weighing less than 60 grammes.

Each treatment was applied to 4 replicate plots. In the first two trials the weight of yield per plot was adopted as unit of calculation; in the last two trials, however, the stand was not quite uniform and we therefore counted the plants per plot and calculated the yield in terms of weight of yield per plant. The significant difference between the weight of yield and the percentage of tubers in grade A under the various treatments was calculated by the analysis of variance, as adapted by SAUNDERS (4), to the level of $P=0.05$.

The incidence of powdery mildew was estimated by the following scale of categories and marks:

	marks
A. The plants are free from powdery mildew	0
B. Trace — isolated lesions on a few stalks	0.1
C. Slight infection — two or more lesions on several stalks	0.5

D. Slight to moderate infection — several lesions on most stalks 1.0
E. Moderate to severe infection — most stalks markedly infected, slight infection of the leaves 2.0
F. Severe infection — stalks covered entirely by powdery mildew, the leaves begin to dry up 4.0
G. Very severe infection — advanced or total drying up of the entire plants 8.0

Most of the plants in each plot were examined and the plot as a whole was then assigned the appropriate mark. The average of the 4 separate marks thus assigned to the 4 replicate plots under each treatment was taken to indicate the fungicidal efficacy of that treatment. To facilitate comparison, the average disease rating of each treatment was also expressed as percentage of the average disease rating of the control plots.

III. THE TRIALS AND THEIR RESULTS

Trials Nos. 1 and 2.

Trial No. 1 was carried out at Kinneret, at the southern end of the Lake of Tiberias, on potatoes sown in late December 1941 on medium soil. Each replicate plot measured 29 sq. metres. The rate of spraying averaged 250 litres per dunam. Powdery mildew was first recorded in late February, about 2 months before the crop was lifted, and the incidence of disease was estimated twice, on 16th and 31st March 1942.

Trial No. 2 was carried out at Tel Josef, in the eastern part of the Valley of Esdrælon, on potatoes sown in mid-December on medium-heavy soil. Each replicate plot measured 48 sq. metres. The rate of spraying averaged 200 litres per dunam. Powdery mildew was first recorded on 20th March, about 5 weeks before the crop was lifted, and the incidence of disease was estimated on 9th April 1942.

Details of the treatments tested, the dates of application, and the effect of treatments on the incidence of powdery mildew and on the size and grade composition of yield appear in table I.

The table indicates that spraying with Sulfinette at 1.5% strength was most effective in controlling the powdery mildew, whereas the copper sprays tested were limited in their effect on the disease. While the powdery mildew attack was still slight, spraying with Bordeaux mixture 1% or with the combined Perenox - white oil spray was somewhat more effective than spraying with Cuprogreen Concentrated or Perenox alone (cf. results of the first estimation in trial No. 1 and of the only estimation in trial No. 2); however, when infection by powdery mildew assumed more serious proportions, these differences in the action of the various copper sprays tested were no longer apparent (cf. results of the second estimation in trial No. 1).

In trial No. 1 treatments Nos. 2 and 8 served to compare the effect on powdery mildew of spraying with Sulfinette at various intervals. Plots under both treatments were first sprayed on 25th February, just after powdery mildew had first been recorded. Treatment No. 2 was again applied on 4th, 11th, and 24th March, and on 1st April. Treatment No. 8, on the other hand, was applied a second time only after a full month's interval, on 24th March, when the disease was seen to progress swiftly, and again on 1st April. The results of the second estimation made in this trial clearly show that the plots sprayed 3 times during the month of March were practically free from powdery mildew, while on the plots sprayed only once in that month infection was moderately severe.

Spraying with Sulfinette slightly scorched the foliage.

TABLE I

The effect of treatments on the incidence of powdery mildew and on the size and grade composition of the yield

(Each figure represents the average of 4 replicate plots).

Treatment	Dates of application	Incidence of powdery mildew				Size of yield per plot		% of tubers in grade A
		1st estimation		2nd estimation				
		Disease rating	%	Disease rating	%	in kg.	%	
Trial no. 1								
1) Control		3.25	100	6.00	100	34.4	100	35.5
2) Sulfinette 1.5%	25th February, 4th, 11th, and 24th March, and 1st April	0.025	0.8	0.30	5	41.2	120	42.6
3) Bordeaux mixture 1%		0.55	17	3.25	54	31.3	91	32.4
4) Perenox 0.33%		1.53	47	3.50	58	37.4	109	34.2
5) Cuprogreen Concentr. 0.33%		1.50	46	3.75	63	37.7	110	41.0
6) Perenox 0.33% +white oil 1%		1.03	32	3.75	63	33.5	97	27.8
7) Perenox 0.33% +white oil 1%	25th Febr., 4th and 24th March	1.50	46	3.50	58	34.5	100	34.1
8) Sulfinette 1.5%	25th Febr., 24th March, 1st April	0.10	3	2.25	38	36.2	105	31.6
significant differences:						7.98	23.2	10.24
Trial no. 2								
1) Control	—	2.25	100			48.3	100	15.8
2) Sulfinette 1.5%	12th, 27th February, 8th, 16th, 24th March and 5th April	0.00	0			52.4	109	11.7
3) Bordeaux mixture 1%		0.10	4			47.3	98	7.4
4) Perenox 0.33%		0.57	25			50.6	105	10.7
5) Cuprogreen Concentr. 0.33%		0.33	15			57.6	119	13.7
6) Perenox 0.33% +white oil 1%		0.10	4			52.5	109	16.2
7) Perenox 0.33% +white oil 1%	12th Febr., 8th 24th March	1.27	56			57.1	118	15.7
significant differences:						10.37	21.5	6.02

In trial No. 1, spraying with Sulfinette (treatment No. 2) increased the weight of yield by 20 per cent. and somewhat raised the percentage of tubers in grade A; however, in both instances the increase was not statistically significant as compared with the control. The copper sprays in this trial hardly affected the size of the yield or its grade composition.

In trial No. 2, where the attack of powdery mildew developed late in the growing period, the complete control of disease achieved by Sulfinette spraying was not reflected in the yield of the plots under this treatment. On the other hand, two of the copper treatments, which were less effective against the powdery mildew, increased the yield to some extent, though not significantly. In the absence of all other diseases the conclusion thus appears warranted, that in this trial the factor determining the size of yield was not the degree of mildew control — the disease apparently spread too late to cause any damage — but the relative phytocidal effect of the treatments tested.

Trials Nos. 3 and 4.

Both these trials were carried out at Kfar Gil'adi, in the Upper Galilee, on heavy soil.

In trial No. 3, spring potatoes were sown in late March and were sprayed on 1st, 8th, 15th, and 22nd June and on 1st July 1943. Each replicate plot measured 30 sq. metres. Powdery mildew was first recorded on 20th June, one month before lifting, and the incidence of disease was estimated on the day on which the crop was lifted.

In trial No. 4 autumn potatoes were sown on 10th September, and the treatments were applied on 7th, 15th ,and 23rd November, and on 1st, 8th, and 15th December 1943. Each replicate plot measured 38 sq. metres. Powdery mildew was first recorded in early November and was estimated on 9th December 1943, about three weeks before the crop was lifted. In addition to the comparative examination of various fungicides, this trial also served to test a novel way of applying sprays, viz. through the overhead irrigation pipes. For this purpose the outlet pipe of a small motor sprayer (3 HP) was linked directly with an overhead irrigation pipe of the revolving type. The pipe had a diameter of 1/2 inch, was 16 metres long, and had 20 nozzles .The spray was applied to plants on either side of the pipe by revolving the latter slowly around its own axis. Further details of this method have recently been described elsewhere (3). The rate of spray application through the revolving pipe was almost twice as heavy as the ordinary rate of spraying.

Details of the treatments tested and their effect on the incidence of powdery mildew and on the size and grade composition of the yield appear in table II.

In both trials the development of powdery mildew was weak. Spraying with Cita Lime-Sulphur Spray, at 1.5% strength, and dusting with Gaza sulphur Extra Fine grade suppressed the disease almost entirely, and spraying with Perenox and Spersul was also effective. The action of the copper spray Perenox was definitely weaker than that of the sulphur treatments.

In trial No. 3, sulphur treatments first applied after appearance of the first symptoms of powdery mildew were seen to control the disease effectively. In trial No. 4, the application of lime-sulphur spray through the overhead irrigation pipe was as effective as spraying in the usual way.

TABLE II

The effect of treatments on the incidence of powdery mildew and on the size and grade composition of the yield

(Each figure represents the average of 4 replicate plots).

Treatment	Incidence of powdery mildew		Size of Yield per plant		% of tubers in grade A
	Disease rating	%	in kg.	%	
Trial no. 3					
1) Control	1.50	100	0.59	100	49.8
2) Perenox 0.33%	0.43	29	0.56	95	47.2
3) Perenox 0.33%, later Perenox 0.33% Spersul 0.5%*)	0.15	10	0.54	92	52.6
4) Perenox 0.33%, later Cita Lime-Sulphur Spray 1.5%*)	0.05	3	0.53	90	48.6
5) Cita Lime-Sulphur Spray 1.5%	0.05	3	0.58	98	55.0
significant differences:					6.89
Trial no. 4					
1) Control	0.88	100	0.55	100	74.2
2) Gaza sulphur Extra Fine Grade	0.00	0	0.59	107	81.3
3) Perenox 0.33%	0.30	34	0.55	100	77.9
4) Perenox 0.33% + Spersul 1%	0.17	19	0.60	109	70.1
5) Cita Lime-Sulphur Spray 1.5%	0.05	6	0.61	111	78.5
6) Cita Lime-Sulphur Spray 1.5% applied through overhead irrigation pipe	0.05	6	0.64	117	80.0
significant differences:			0.104	19.0	17.52

*) The plots under treatments nos. 3 and 4 (trial no. 3) first received two applications of Perenox and later, after appearance of the powdery mildew, three applications of their respective sulphur sprays.

Spraying with Cita Lime-Sulphur Spray caused some scorching, and this was particularly noticeable on the plots to which this spray was applied at a heavier rate through the overhead irrigation pipes.

In trial No. 3 none of the treatments increased the yield, and in trial No. 4 the yield increases effected ranged from 7-17%, as compared with the control, and were not significant. The effect of treatments on the grade composition of the yield was likewise too small to be significant.

IV. DISCUSSION

Fungicidal action. — In 1921, DUCOMET (1) observed in France that copper solutions used in the treatment of blight also appeared to check a powdery mildew disease of potatoes, surmised to be caused by *Erysiphe tichoracearum* or an allied form. This was confirmed in our trials, where all the copper sprays tested reduced the incidence of powdery mildew to some extent. Under conditions of slight infection, Bordeaux mixture (1%) was somewhat more effective than the proprietary fixed copper sprays Perenox (0.33%) and Cuprogreen Concentrated (0.33%); however, when conditions favoured a heavier outbreak of powdery mildew, these differences in the action of the various copper sprays were no longer apparent and they all achieved partial control only.

The addition of light-medium white mineral oil appeared somewhat to increase the action of Perenox on the powdery mildew, where the incidence of disease was slight, but was of little advantage under conditions of severe infection. MARTIN & SALMON (2) have reported on the fungicidal action of some vegetable oils in the control of hop mildew (*Sphaerotheca humuli*); our results appear to indicate a similar, though slight, effect of the mineral oil on the potato mildew, but our data are insufficient to state such effect with certainty.

However, the effects of the various copper sprays, with or without addition of oil, were insignificant if compared with that of the sulphur fungicides tested: the lime-sulphur preparations Sulfinette and Cita Lime-Sulphur Spray, both at 1.5% strength, suppressed the development of powdery mildew on potatoes almost entirely, and in the last trial dusting with Gaza sulphur Extra Fine grade was equally effective. The action of the sulphur preparation Spersul, when added to the Perenox spray, was also satisfactory, but the optimum concentration of this material has yet to be determined.

2. *Overhead application.* — The application of Cita Lime-Sulphur Spray through the overhead irrigation system controlled the powdery mildew on potatoes as effectively as applications of the same spray made in the usual fashion, by means of power sprayers. The advantages of spray application through the overhead irrigation system are being discussed elsewhere (3).

3. *Phytocidal action.*—The two lime-sulphur sprays tested, Sulfinette and Cita Lime-Sulphur, slightly scorched the potato foliage when used at 1.5% strength. Scorching was more pronounced on those plots to which lime-sulphur spray was applied through overhead irrigation pipes at a rate considerably exceeding the usual rate of application. However, the scorching was not reflected in any reduction of yields.

4. *Timing and frequency of applications.* Treatments commencing with the appearance of the first symptoms of disease suffice to control the powdery mildew of potatoes, and preventive treatments are not necessary. This point is of considerable importance, because the occurrence of the disease in Palestine has so far been sporadic and the inclusion of sulphur treatments in the scheme of preventive potato treatments would much increase the labour and material outlay.

As regards the frequency of applications, trial No. 1 has shown that the effect of lime-sulphur spraying lasts for at least two weeks. It may therefore be assumed that the frequency of applications will have to be decided in accordance with (a) the rate of growth, i. e. the amount of new and unprotected foliage produced in the treatment intervals, and (b) the amount of rainfall or of overhead irrigation liable to wash the spray or dust residue from the leaves and haulms. In general, applications made at 10-14 days' intervals may be assumed to afford full protection.

5. *Yields.* — The trials show that yields are not appreciably reduced if potatoes are attacked by powdery mildew during the last month of growth. In this respect powdery mildew resembles *Alternaria* blight. But when the crop is attacked at an earlier stage of growth, powdery mildew may reduce the yield by 20 per cent. or more and the grade composition of the yield is also affected adversely. These findings warrant the conclusion that measures for the control of powdery mildew should be applied over a period beginning with the appearance of the first symptoms of disease and ending 3 to 4 weeks before the crop is lifted.

V. SUMMARY

(1) The occurrence in Palestine is reported of a powdery mildew disease (*Oidium sp.*) attacking potatoes. The symptoms of the disease are briefly described.

(2) Means of combating the disease were tested in four trials. Lime-sulphur sprays (Sulfinette and Cita Lime-Sulphur), used at 1.5 per cent. strength, were found to suppress the development of powdery mildew almost entirely. The dispersible sulphur spray Spersul, used in combination with Perenox, was also effective. In one trial, dusting with Gaza sulphur (Extra Fine grade) gave equally satisfactory results.

(3) All the copper sprays tested (Bordeaux mixture, Perenox, Cuprogreen Concentrated) were much weaker in their effect on the mildew. The addition of light-medium white oil to the Perenox spray slightly increased the effect of the latter.

(4) The application of lime-sulphur sprays through the overhead irrigation system succeeded, in one trial, in checking powdery mildew as effectively as sprays applied in the usual way.

(5) The lime-sulphur sprays (1.5 per cent.) slightly scorched the potato foliage.

(6) Treatments for the control of powdery mildew should begin at the appearance of the first symptoms of disease, and should be repeated at 10-14 days' intervals until 3 to 4 weeks before the crop is lifted.

(7) Powdery mildew reduces the yield only if the attack occurs during the first 2 months of growth. Yields may then be decreased by 20 per cent. or more, and their grade composition is also adversely affected.

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THE OCCURRENCE OF APPLE AND PEAR SCAB IN PALESTINE IN RELATION TO WEATHER CONDITIONS

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The scab disease of apples and pears, caused by the fungi *Fusicladium dendriticum* (Hall.) Fuck. and *F. pirinum* (Lib.) Fuck., occurs in most countries in which these crops are grown and is also found in Palestine. The fungi have so far been found in this country only in their imperfect stage.

Observations over a number of years have shown that the only apple and pear varieties affected by scab in Palestine are the local ones, while varieties recently introduced from Europe remain healthy. This is true even where Palestinian and European varieties are growing side by side on the same plot or in alternate rows. Surprising though this may at first appear, a comparison of the ecological requirements of the scab fungi with weather conditions conducive to the growth of local and European varieties will explain this phenomenon.

Keitt and Jones (1) in their detailed experiments on the biology of *Fusicladium*, confirm the observations of other writers that the development of scab is conditioned by various environmental factors pertaining to the host and parasite. These authors found a temperature within the limits of 6°C to 26°C to be prerequisite to infection by *Fusicladium*. Even within these temperature limits high atmospheric humidity does not suffice to bring about spore germination, but the spores have actually to be immersed in a film of water before they will germinate. The period of immersion required for spore germination or leaf infection varies with temperatures. At 6°C the spores germinate and cause the first signs of infection after 13-18 hours, at 9°C after 9-11 hours, at 15°C after 5-8 hours, at 20-24°C after 4-6 hours, and at 26°C after 8-10 hours. At higher temperatures the time required for germination lengthens, and at 32°C spore germination ceases entirely even in water.

The age or degree of maturity of the host is a further factor bearing on scab development, young leaves and fruits being more susceptible to infection than older ones. The time in which epidemic dissemination of and infection by scab is possible is therefore limited to a short period during and after blossom and leaf formation.

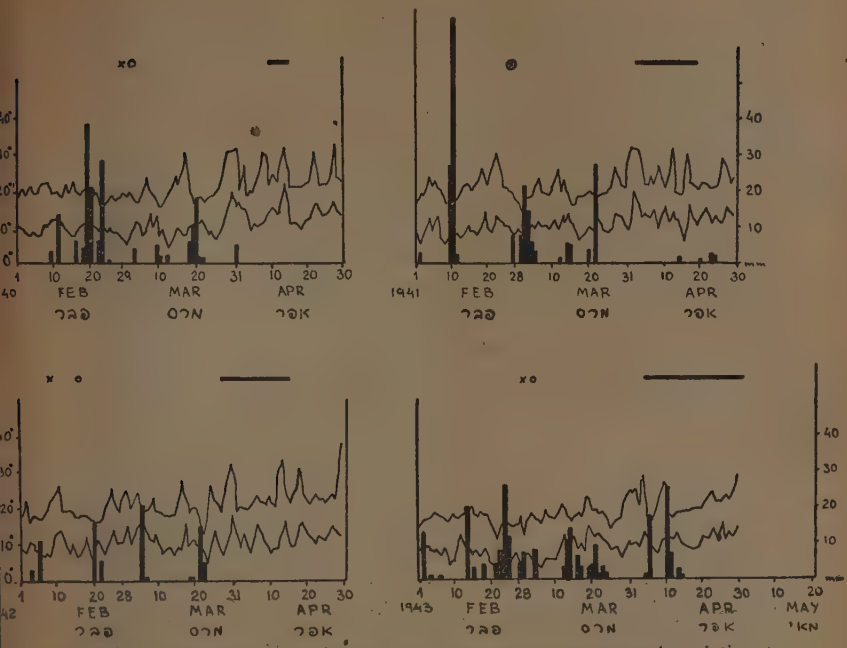
The above mentioned conditions favouring the development of scab exist in Palestine only in winter, up to the month of March. In winter, rain is abundant and the leaves are wet during periods in which temperature does not exceed 25°C. From March onwards the rains cease and later rainfall occurs only in exceptional years. If there are rains, their effect is slight because moisture on the leaves dries rapidly. Temperatures in spring increase swiftly. Minimum temperatures may then approximate 10°C, while maximum temperatures may rise to 35°C. The highest temperatures of the year occur in March and April. In most years, dry east winds (khamsins) are prevalent during these months for a few days at a time, when temperature reaches its annual maximum and atmospheric humidity its annual minimum.

A comparison of the dates of leaf and blossom formation of the apple and pear varieties grown in this country shows that local apple varieties, such as Hashabi and Biari, blossom and sprout in the rainy period, in late February to early March, while European apple varieties, such as Grand Alexander, Rome Beauty, Winter Banana, Peasgood, Delicious, Red Astrachan, and others, begin to form their leaves only in late March or early April, at any rate not before late March. The interval between the date of blossoming of the local and the above European varieties approximates 4 to 6 weeks. During that period the weather changes from rainy with low temperatures to dry and rainless accompanied by high temperatures.

Since local varieties form leaves and blossoms during a period when weather conditions are favourable to the development of the scab fungus, these varieties are in some years infected, and the incidence of disease may even be severe. The European varieties, on the other hand, are not infected as they blossom later in the season, when weather conditions are unfavourable to the germination of spores. The period in which the latter varieties form their leaves is devoid of humidity and temperatures approach or sometimes exceed the maximum at which the scab fungus ceases to develop.

A graph showing the relative dates of blossoming, during 1940-1943, of the above mentioned local and European varieties in the orchard of the Government Agricultural Experiment Station, Acre, is reproduced in tex-fig. 1, where amounts of rainfall and maximum and minimum temperatures during the months of February to April are also indicated. The graph clearly bears out what has above been stated in general terms, that the respective dates of blossoming of local and European varieties were more or less constant over a period of four years, and differed from one another by 4 to 6 weeks.

Similar relations apply in other parts of Palestine. Exact records of the blossoming dates of local varieties over a longer period (5 years) were made only at Acre. In the Nablus district similar records were available only for 2 years, and in the Jordan Valley

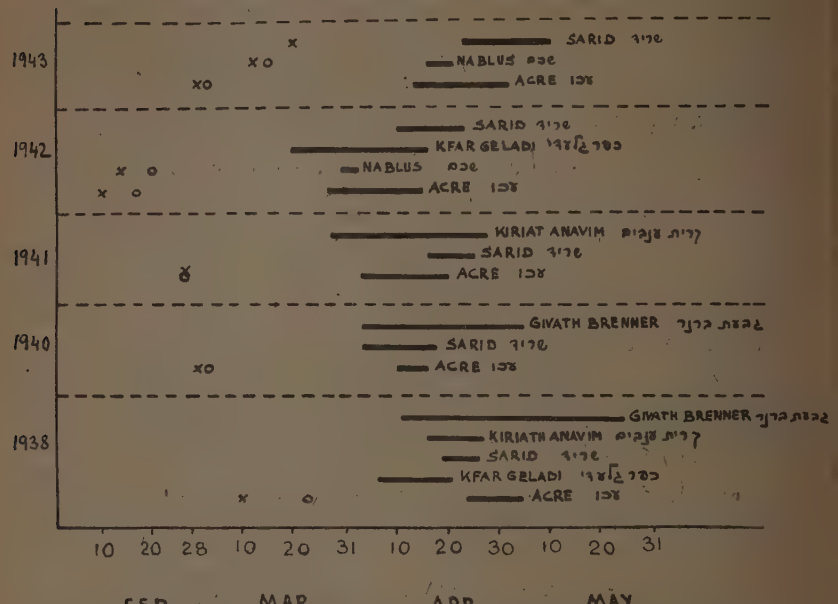


Text-fig. 1. Rainfall, and maximum and minimum temperatures in relation to the date of blossoming of the local varieties Hashabi (x) and Biari (o) and of European apple varieties (—) at Acre during 1940-1943.

(Degania) and the Nazareth district (Sarid) only for one year. These records are represented in text-fig. 2 and support the conclusion that the European varieties mentioned above blossom from 31 to 45 days after the local varieties. In all these instances the blossoming dates of the local varieties occur at a period of rainfall and low temperature, while those of the European varieties occur in a dry, rainless period, when temperatures are much higher than in late February or early March. Even in so unusual a year as 1943, when the rainy season was abnormally late and local varieties formed their blossoms and leaves during a period of abundant rains, the European varieties blossomed only during the last spells of rain which were at once succeeded by warm and dry weather.

The data presented above with regard to the local apple varieties Hashabi and Biari are applicable to a large extent to pear varieties as well. Although exact records are not available, numerous observations have shown the local pear varieties, such as Baladi, to form their leaves and blossoms during the rainy season, while European varieties such as Superfine, Bartlett, Amanlis, New Poiteau, and

others blossom later, when the weather is drier. However, with pears the interval between the blossoming of local and European varieties is shorter than with apples and amounts to approximately 2 to 3 weeks. There is no doubt that only local varieties, especially Baladi, but none of the European varieties, suffer from scab attack.



Text-fig. 2. The date of blossoming of the local apple varieties Hashabi (x) and Biari (o) and of European apple varieties (—) in various districts of Palestine.

Apart from typical local varieties blossoming early in the season, such as Hashabi and Biari apples and Baladi pears, there are other varieties which might be local, but their origin is doubtful and they blossom later than the above mentioned local, but earlier than the European varieties. These types are exemplified by Sukri apples and Ein Harodi pears. As might be expected, these and similar varieties are infected with scab only when rain is abundant and continues to fall late in the season.

The evidence presented here entirely confirms the conclusions reached by many authors, especially KEITT and JONES (1) and MOORE (2), that the appearance of scab depends on weather conditions. This appears to be the first record of circumstances under which European varieties originally susceptible to scab seem to acquire resistance by transfer to another country. In reality, of course, the susceptibility

of these varieties does not change but — owing to the prevalence during blossom and leaf formation of weather conditions unfavourable to scab development — they escape the disease.

Acknowledgment. The author is obliged to A. Goor, Esq., Senior Horticultural Officer of the Department of Agriculture, Government of Palestine, and to Dr. J. Carmon, Head of the Division of Horticulture, Agricultural Research Station, Rehovot, who placed at his disposal the records of blossom and leaf formation of the different varieties in various localities. Dr. D. Asiibel, of the Hebrew University, Jerusalem, was kind enough to supply the meteorological data.

SUMMARY

The scab disease of apples and pears, caused by the fungi *Fusicladium dentriticum* and *F. pirinum*, occurs in all parts of Palestine and affects the leaves and fruits.

The diseases have so far been found only on local varieties of apples and pears. These varieties form their blossoms and leaves early in the season, when low temperatures prevail and rains are frequent. The European apple varieties introduced into Palestine in recent years commence their yearly growth 4-6 weeks later, and the European pear varieties 2-3 weeks later, when rains have usually ceased to fall, the temperature is higher, and atmospheric humidity low. According to Keitt and Jones (1), spores of *Fusicladium* will germinate only when actually immersed in water and at low temperatures, and the fungus is liable to infect leaves and fruits only where these conditions are fulfilled. As this is not the case when European varieties form their leaves and blossoms, these varieties escape infection.

Thus European apple and pear varieties in Palestine remain free from scab attack not owing to their resistance to the disease, but owing to weather conditions.

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PATHOGENICITY OF *DIPLODIA* FROM VARIOUS HOSTS TO CITRUS FRUITS

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Numerous plants common in Palestine, in the genus *Citrus* as well as in other genera, are known to be affected by one or more species of *Diplodia* resembling that causing blight and fruit rot of Shamouti oranges and of other species of *Citrus* (*D. natalensis* Pole Evans). The various species of *Citrus* are often interplanted, and many of the other hosts are grown in the vicinity of citrus groves, some serving as wind breaks (castor bean) or hedges (*Acacia farnesiana*) around groves, while others, such as date palm and mulberry or walnut trees, are sometimes actually found in citrus groves. It was therefore of interest to determine the pathogenicity of *Diplodia* from one species of Citrus to other species of genus and to ascertain whether *Diplodia* from other hosts may induce stem end rot of citrus fruits.

A culture mash of Diplodia from each host was inoculated into the base of 5-10 citrus fruits from which the buttons were first removed; the wound was subsequently sealed with paraffin. In a few cases, where dry spores were available, a suspension of spores was also used for inoculation. The fruits were then incubated at a temperature favourable for the development of *Diplodia*, i. e. at 18 to 25°C.

I. CROSS INFECTIONS WITHIN THE GENUS *CITRUS*

Diplodia natalensis in Palestine causes twig blight of the following species of *Citrus*:

Shamouti orange (*Citrus sinensis* Osb. Shamouti), Valencia orange (*C. sinensis* Osb. Valencia), lemon (*C. limonia* Osb.), grapefruit (*C. paradisi* Macf.), mandarine (*C. nobilis* Lour. var. *deliciosa* Swingle), sweet lime (*C. aurantifolia* Swingle var. *dulcis*), and bitter orange (*C. aurantium* L.). *Diplodia* has been found to cause stem-end rot on fruits of all these species, except sweet lime and bitter orange.

As stated above, the different species of citrus fruits are often planted in close proximity in the groves; sweet lime and bitter orange serve as stocks for all the species. As spores from twigs or branches affected with blight represent the principal source of infection for stem-end rot, tests were therefore made with the above

species, except sweet lime, to determine if inoculum from the twigs of one may induce stem-end rot in fruits of other species. For the sake of clearness the scheme of inoculations is below given in tabular form.

Inoculum from twigs of	*Inoculated into fruits of*
Shamouti orange	Shamouti orange, lemon, grapefruit, mandarine
Lemon	Shamouti orange, lemon, grapefruit
Grapefruit	Shamouti orange, lemon, grapefruit
Valencia orange	Shamouti orange
Mandarine	Shamouti orange
Bitter orange	Shamouti orange

All the inoculations, carried out over the 1939/40 - 1941/42 seasons, were successful. It was thus proved that *Diplodia* from twigs of Shamouti orange, lemon, or grapefruit may cross-infect the fruits of any of these three species; that inoculum from Shamouti orange twigs may induce rotting of mandarine fruit and vice versa; and that *Diplodia* from twigs of Valencia and bitter orange may infect Shamouti orange fruits.

II. INFECTION OF CITRUS FRUITS FROM HOSTS OUTSIDE THE GENUS *CITRUS*

In tests carried out from 1939/40 to 1943/44, *Diplodia* isolated from the following sources was capable of inducing rot in Shamouti oranges as rapidly as *D. natalensis* from blighted citrus twigs or from oranges affected with stem-end rot:

Acacia farnesiana	trunk (bark)
Arachis hypogaea, ground nut	stalk
Cereus Straussii	root
Cydonia oblonga, quince	root
Eriobotrya japonica, loquat	twigs
Euphorbia grandidens	shoots
Ficus elastica	branches
F. nitida	branches
Juglans regia, walnut	branches
Malus communis Hashabi, apple stock	root collar
M. mitis (Doucin), apple stock	root collar
Mangifera indica mango	twig and fruits
Morus alba, mulberry	branches

M. rubra, mulberry	branches
Musa sp. (Cavendishi), banana	fruits
Prunus Myrobalan, plum stock	root collar
P. Karassya, plum stock	root collar
Pyrus malus, apple	trunk and fruit
Ricinus communis, castor bean	stem
Rosa sp., rose	collar and rots
Vitis vinifera, vine	fruit stalk

Diplodia from *Mentha piperita* (in 2 years' tests), and from *Pyrus syriaca* (one year) failed to induce orange rot. Inoculum from date palm (*Phoenix dactylifera*), from two separate sources, in 3 years' tests, induced rotting only in isolated instances after very long periods of incubation.

Further inoculations of grapefruit and lemon fruits were made in one year with *Diplodia* from castor bean, *Acacia farnesiana* and date palm. Inoculations from the first two sources were successful, but *Diplodia* from date palm failed to induce stem-end rot of these fruits.

The negative results obtained with *Diplodia* from date palms are in agreement with the findings of FAWCETT and KLOTZ (1), who referred the *Diplodia* on this host to the species *D. phoenicum* (Sacc.).

Diplodia was found in abundance on castor beans and *Ficus nitida*. Pruned twigs of these hosts were observed to form a suitable medium for the rapid development and dissemination of the fungus under humid weather conditions.

Acknowledgement. Thanks are due to Dr. M. CHORIN and Dr. J. PERLBERGER, of the Division of Plant Pathology, for several of the isolates of *Diplodia* used in this study.

CONCLUSIONS

The above investigations have shown that *Diplodia* stem-end rot of Shamouti orange fruits may be induced by spores not only from twigs of all the other species of *Citrus* tested, but also from almost all the hosts outside the genus *Citrus*, with the exception of date palm, *Mentha piperita* and *Pyrus syriaca*. *Diplodia* from castor bean and *Acacia farnesiana*, but not from date palm, induced stem-

end rot of grapefruit and lemon fruits. *Diplodia* from twigs of any one of the species of *Citrus* grown in Palestine was seen to be capable of causing stem-end rot of each of the three most important citrus fruits (oranges, lemons, grapefruits). In the light of these results *Diplodia* from almost any source must be considered a potential danger to oranges.

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ANATOMICAL STUDY OF THE BUTTON OF SHAMOUTI ORANGES IN RELATION TO STEM-END ROT

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(With 1 Text-figure)

INTRODUCTION

The stem-end rot of oranges, which in Palestine is so prevalent in storage, is chiefly caused by *Diplodia natalensis* Pole Evans, and to a minor degree by *Alternaria sp.* and *Colletotrichum gloeosporioides* Penz. These rots are not usually apparent while the fruit is still on the tree, but develop only after picking. As indicated by their name, the rots begin at the stem-end of the fruit and the fungi are commonly assumed to penetrate the fruit through the button (2, 5, 6, 7, 9). It was, however, not clear whether, when the fruit is being picked, the organisms causing stem-end rot are only superficially present on the button or whether they are already established within its tissues. It was likewise unknown, which part of the button actually harboured the pathogens. An anatomical study of the button of Shamouti oranges was therefore made to contribute towards the elucidation of these questions.

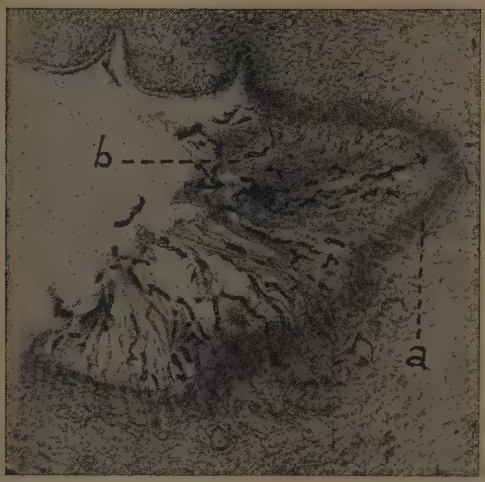
Buttons fixed in alcohol-formalin-acetic solution were cut by freezing microtome and stained with cotton blue and sometimes with Pianeze solution.

PATHOLOGICAL ANATOMY OF THE BUTTON

No mycelium was ever found to be present in the inner tissues of any part of the button of newly picked oranges. On the other hand, mycelium was found to be superficially present on various parts of the button, occurring as a rule in the axil of sepals and sometimes in form of lesions on other external parts of the button.

The occurrence of mycelium was always accompanied by the presence of a special tissue consisting of 5-7 dense rows of flat, elonated cells of the yellow colour typical of cork layers. Histochemical tests (KOH, Sudan III) confirmed the presence of cork in the cell walls. No mycelium was at any time detected in the cork layer itself or in the deeper button tissues beyond and inside this layer.

The cork layer formed in the axil of sepals, near the receptacle, was found to be characteristic in appearance. On its outer side this layer passes into necrotic, torn, and deeply indented, finger-like tissue (text-fig. 1). It was in this necrotic tissue that mycelial threads, usually brown but occasionally hyaline in colour, were generally present, sometimes together with spores.



Text-fig. 1. Section through axil of sepal on the button of Shamouti orange: (a) cork layer; (b) necrotic tissue containing mycelium. (× 100).

In order to determine the stage at which necrotic tissue and cork layer form in the axil of the sepals, and to elucidate the function of the cork layer, we examined the buttons at various stages of fruit development, first in the flowers, then in young fruits shortly after they had set, and subsequently throughout the period of fruit ripening.

Necrotic tissue as well as cork layer were found to be absent in flowers. The cork layer may be assumed to form as a protective tissue (wound cork) after petals and stamens have shed and was already seen to be present in fruits measuring from 1 cm upwards in length. Further observations showed this cork layer to form some distance below and around the point at which the petals and stamens are detached. The tissue between this point and the cork layer becomes necrotic, the cells near the cork layer remaining more or less dense, while the outer tissues are deeply torn and assume a finger-like configuration. It was in this necrotic tissue that mycelium, and sometimes spores, were usually encountered even in very young fruits measuring from 3 cm upwards in length. This tissue may therefore be assumed to serve as shelter for the spores until conditions are

favourable for their germination. After germination, the mycelium may penetrate between the cells of the necrotic tissue until it reaches the dense cork layer beyond which it has never been detected on fruit still attached to the tree. The cork layer thus acts as a barrier preventing deeper penetration of the fungi.

As mentioned above, protective cork layers associated with mycelium and similar to that described above as occurring in the axil of sepals, were also observed on other parts of the button. Such layers were sometimes found on large portions of the upper and lower surface of sepals, less frequently on the outer surface of the receptacle (discus) and on the fruit stalk, but often at the tip of the sepals. The fact that mycelium and cork layer are almost invariably found to be present at the tip of sepals of mature fruits can possibly be explained as follows: young sepals are covered at their tip by hairs which might well harbour spores; the latter subsequently germinate, the mycelium penetrates deeper into the tissues, and a cork layer forms below and around the point of infection. The epidermis disappears wherever lesions containing mycelium and underlain by a cork layer form on the button.

FORMATION OF THE CORK LAYER

The conditions under which such cork layers form in various parts of the button, whether in response to either fungal penetration or mechanical wounding or to a combination of the two, were investigated in the following experiment, full details of which are given elsewhere (4): In September young fruits attached to the trees were wounded by pricking with a sterile needle only or by pricking followed by inoculation with a suspension of *Diplodia* spores. Pricks were applied (a) to the fruit stalk, close to the button, to the depth of the peel; (b) to the upper surface of the sepals, and (c) to the lower surface of the sepals. Additional inoculations, without wounding, were made by the application of a spore suspension to the entire button by means of a soft brush. The fruits were picked 8 months later and their buttons were then examined. Cork layers were found to have formed around all the pin pricks, irrespective of subsequent inoculation. On the button of those fruits to which spores had been applied by brush, mycelium had at some points developed in place of the epidermis; but this mycelium was always separated from the healthy inner tissue by a cork layer, and never extended beyond the latter. These observations indicate that cork layers form on the button of orange fruits either as healing layers after petals and stamens have shed or around wounds, or in response to, and prevention of, the penetration of fungi into the living tissue of fruit still attached to the tree.

THE FUNGI PRESENT ON THE BUTTON

In view of the fact that only mycelium was in general encountered in the above anatomical examinations, there arose the question which fungi this mycelium belonged to. Further sections were therefore made of one half of buttons, the other half of which was cultured in a separate set of experiments (3). Examinations were carried out only on the halves of those buttons which, on their other halves, yielded but a single fungus (either *Diplodia* or *Alternaria* or *Colletotrichum*) in cultures made after disinfection.

In these examinations, just as in those described above, mycelium was never found to be present in the inner tissues of the buttons, but only in the necrotic tissue in the axil of sepals and, superficially, on some of the other parts of the button mentioned previously. We may thus infer that the mycelium observed on various parts of the button may well belong to one of the fungi causing stem-end rot which appeared in the corresponding cultures (*Diplodia, Alternaria, Colletotrichum*).

CONCLUSIONS

Various writers, including BAKER (1) and SIMMONDS (8), hold that the presence of mycelium in the outer tissues of fruits constitutes a source of latent infection. Our observations likewise indicate that the occurrence of mycelium on various parts of the button may be the source of such latent infection of the stem-end rots of Shamouti oranges caused by various fungi, and especially by *Diplodia natalensis*, during storage and transit. These fungi appear to be unable to penetrate the cork layer formed regularly in the axil of the sepals and sometimes in other parts of the button, and to infect the fruit on the tree. This may be the reason why *Diplodia* rot is very rarely found to occur on fruits before they have been picked. After picking, on the other hand, the mycelium of fungi present at various points in the outer tissues of the button appears to penetrate the button of fruits, in a manner not yet adequately understood, and to cause storage rots.

SUMMARY

An anatomical study was made of the button of Shamouti orange fruits to determine where the fungi causing stem-end rot after picking are harboured.

No mycelium was ever found to be present in the inner tissues of the button of picked fruit, but it was seen to occur in the axil of sepals, within the torn, necrotic tissue forming at the point at which petals and stamens have previously been attached, and sometimes also on other external parts of the button.

The occurrence of mycelium on these outer parts of the button was always accompanied by the presence of a cork layer separating the necrotic tissue and mycelium from the healthy tissue of the button. This cork layer was shown to form in response to mechanical injury (pin pricks) as well as to fungal invasion.

Further observations carried out in conjunction with cultural studies permit the inference, that the mycelium found superficially on the button belongs to fungi causing stem-end rot (*Diplodia natalensis, Alternaria sp.* or *Colletotrichum gloeosporioides*). The presence of such mycelium therefore constitutes a source for latent infection of these rots in storage.

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TRIALS FOR THE CONTROL OF COVERED SMUT OF BARLEY BY SEED DRESSINGS

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The covered smut disease of barley (*Ustilago hordei* (Pers.) Kell. et Sw.) is widespread in all parts of Palestine and is the principal cause necessitating the annual disinfection of barley seed. Earlier work (4,5) has shown that seed steeping treatments and some of the dry mercurial seed dressings practiced all over the world afford good control of this disease under Palestinian conditions.

During the last decade numerous attempts have been made to replace the mercurials by non-poisonous and cheaper seed dressings. JONES (1) reported good control of covered smut of barley by the use of sulphur dust, and his results were confirmed by PETIT (3). As sulphur costs only a fraction of the cost of mercurial seed dressings, these investigations were of considerable interest. The series of trials described here was therefore primarily designed to test the efficacy in the control of barley smut of sulphur dust as compared with mercurial dusts of known repute, such as Agrosan G. In addition, the effect of sprinkling the barley seed with a concentrated mercurial solution was tested in some of the trials, the local product Caspan being used for the purpose.

The authors wish to express their gratitude for the help extended to them from many sides in the execution of these trials.

MATERIAL AND METHODS

The seed used in trials Nos. 1, 2, and 4 was derived from fields naturally infected with covered smut, but the seeds were not artificially inoculated with smut spores. In trials Nos. 3, 5, and 6 the seed was inoculated by dusting with smut spores at the approximate rate of 1 gr. of spores per kg. seed.

In all the trials, with the exception of No. 2, the size of replicate plots was 25 to 30 square metres, and each treatment was replicated 3 to 6 times. The seeds were always sown by broadcasting.

The efficacy of treatments in the control of covered smut was determined by counts of the number of affected plants on each plot. Only in trial No. 5 was the incidence of disease so severe that it sufficed to take counts on a belt of 1 metre's width traversing the

centre of each plot along its whole length. The number of affected plants, rather than of affected ears, was adopted as standard of comparison because every plant infected by covered smut — even if it produces a number of smutted ears — indicates the failure of the disinfectant to give adequate protection to a single seed only.

The results of all those trials in which treatments were replicated 4-6 times have been analyzed by 'Saunders' (6) adaptation of the analysis of variance to the level of P=0.05.

RESULTS

Particulars of the treatments tested and the results of the counts of smutted plants appear in table I. This table shows that the trials were carried out under widely varying conditions. In trials Nos. 1 and 2 conditions were obviously unfavourable for the development of covered smut and the disease developed even on the control plots on only a small number of plants. In trials Nos. 3 and 6, the incidence of disease is seen to have been moderately heavy, but it was severe in trials Nos. 4 and 5. Particularly favourable conditions appear to have obtained in trial No. 5, where approximately 8 per cent. of the control plants were found to be infected.

Under these widely divergent conditions the action of some of the treatments tested varied considerably. Consistently good results was in all cases given only by Agrosan G. Used at the rate of 2 gr. per kg. seed, this dust almost entirely suppressed the development of covered smut even under conditions conducive to severe infection.

Yellow sulphur dust, on the other hand, was not uniformly successful in controlling the smut. Under conditions of light infection (trial No. 2) sulphur equalled the action of Agrosan G, but where conditions permitted moderately heavy (trial No. 3) or heavy (trial No. 4) infection, the action of sulphur was unsatisfactory. In these trials sulphur was applied at the rate of 4-5 gr. per kg. seed. In the trials carried out in 1942/43 we attempted to raise the efficacy of the sulphur treatment by increasing the rate of application to 8 gr. per kg. seed. However, even this increased dose failed to give results comparable with those obtained with the use of Agrosan G. This is clearly brought out by the results of trials No. 5 (severe infection) and No. 6 (moderately heavy infection). The results of all these trials warrant the conclusion that sulphur dust fails to give adequate protection against covered smut of barley where conditions are very favourable for the development of this disease; the use of sulphur as a seed dressing for barley is therefore restricted to conditions of light infection, i. e. where the seed is known to derive from a field more or less free from covered smut.

The effect of the sprinkling treatment was always inferior to that of the dry seed dressings, even when the strength of the solution was raised to 5% (trials Nos. 5 and 6).

TABLE I

Control of covered smut by seed treatments

Trial No.	Variety	Treatment and rate of application per kg. seed	No. of replicates	Covered smut: mean no. of plants infected per plot	Covered smut: mean no. of plants infected per dunam	Covered smut: % infection in relation to control
	1940/1941					
1	six-rowed	Control -	3	7	200	100
		Agrosan G 2 gr.		0	0	0
		Yellow sulphur 4 gr.		0	0	0
2	six-rowed	Control -	2	27	27	100
		Agrosan G 2.5 gr.	2	0.5	0.5	1.9
		Yellow sulphur 5 gr.	1	0	0	0
	1941/1942					
3	six-rowed	Control -	4	43	1495	100
		Agrosan G 2 gr.		0.5	17	1.1
		Yellow sulphur 4 gr.		3.5	122	8.2
		Caspan *)		6.3	217	14.5
	significant difference between control and disinfectant treatments:			5.25	183	12.2
	significant difference between disinfectant treatments:			4.78	166	11.1
4	two-rowed	Control -	4	77.2	3151	100
		Yellow sulphur 4 gr.		19.5	794	25.3
		Caspan *)		19.5	794	25.3
	significant difference between control and disinfectant treatments:			11.66	476	15.1
	1942/1943					
5	two-rowed	Control -	5	76.4	12,730	100
		Agrosan G 2 gr.		0.2	33	0.3
		Yellow sulphur 8 gr.		13.6	2260	17.8
		Caspan **)		22.8	3800	29.8
	significant difference between control and disinfectant treatments:			10.59	1765	13.9
	significant difference between disinfectant treatments:			4.96	827	6.5
6	six-rowed	Control -	6	16.5	723	100
		Agrosan G 2 gr.		0.2	7	1.0
		Yellow sulphur 8 gr.		1.7	74	10.1
		Caspan **)		4.3	192	26.2
	significant difference between control and disinfectant treatments:			2.16	96	13.1
	significant difference between disinfectant treatments:			1.66	74	10.0

*) sprinkling treatment applied at the rate of 375 gr. Caspan in 1.5 litres water per 50 kg. of barley seed.

**) sprinkling treatment applied at the rate of 75 gr. Caspan in 1.5 litres water per 50 kg. of barley seed.

CONCLUSIONS AND SUMMARY

In six trials for the control of covered smut of barley (*Ustilago hordei*), Agrosan G, used at the rate of 2 gr. per kg. seed, was the only treatment giving consistently excellent results even under conditions conducive to severe infection. The results agree with findings reported abroad (2). Sprinkling the seed with a concentrated mercurial solution (Caspan) gave inferior results.

As regards sulphur, the trials confirmed the results obtained in Egypt (1) and Tunisia (3) in as much as they showed that sulphur may check the development of covered smut. However, our trials proved this effect to be limited. Under conditions of light infection sulphur dust may suffice to control the disease; but where infection is more severe, e. g. where the seed derives from heavily infected fields, the action of sulphur dust — even when applied at the rate of 8 gr. per kg. seed — is unsatisfactory and inferior to that of Agrosan G. Jones' (1) findings that sulphur may generally be recommended for the control of covered smut under all conditions were therefore not substantiated in our trials, and the use of sulphur dust for this purpose is advised only where the seed is known to be reasonably free from contamination with smut spores.

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LEAF AND STEM SPOT OF EGYPTIAN CLOVER

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(With 1 Text-figure)

Egyptian clover (berseem, *Trifolium alexandrinum*), which ranks among the most important forage crops grown in Palestine, has in recent years suffered increasingly from a disease causing spots on leaves and stems and leading to the drying up of the whole plants.

SYMPTOMS

The spots first appear on the lower leaf surface in form of small, round, dark brown, depressed lesions surrounded generally by a lighter yellow halo. After some time, as the lesions grow in size, they are also seen on the upper leaf surface; the spots then assume a

Text-g. 1. Leaf spot of Egyptian clover.

light brown, dry appearance in their centre and concentric zones are sometimes distinguishable there, while the border of the spots remains dark brown in colour, and the halo disappears. The spots first show at the tip, along the veins, and in the centre of the leaf. Individual

spots may unite to form larger lesions and ultimately cause the leaf to dry up. Numerous small, black dots are sometimes visible on the central portion of the spots.

The symptoms of disease on the stems resemble those on the leaves, but the spots are more elongated and may unite to cover the entire stem. The lesions are at times seen to penetrate some way into the stem tissues; the outer tissues of the stem split, and a canker like lesion is thus formed.

DESCRIPTION OF THE FUNGUS

The dots found on the dry spots are black, more less round pycnidia, measuring 90—150μ in diameter. The spores are two-celled, hyaline, and rounded at their ends; they are in general slightly constricted at the septum.

In examinations of about 150 samples collected at various times in 22 localities throughout Palestine the spores were never found to contain more than one septum. As stated above, the mature cells are usually two-celled, but young spores sometimes consist of one cell only. The spores measure 15—20μ by 3—5μ in size.

According to these characteristics the fungus belongs to the genus *Ascochyta*. To determine the species of this pathogen on Egyptian clover, we have compared it, in the table set out below, with the species of *Ascochyta* and related fungi described abroad on clover and lucerne. Species of *Phyllosticta* and *Stagonospora* have been included in the table because on the one hand young *Ascochyta* spores, which have not yet developed a septum, may be confused with *Phyllosticta*, while on the other hand young spores of *Stagonospora*, which do not yet possess more than one septum, closely resemble those of *Ascochyta*. Two species of *Ascochyta* described on the hosts in question have been omitted from the table. One of these is *A. caulicola* Laub. (1,7), which occupies a distinct position because it causes hypertrophy of the host plant. The second is *A. lethalis* Ell. and Barth, described in the United States, which does not resemble our fungus as it seldom occurs on the leaves and, as stated by JONES and WEIMER (6), "fruits rarely on leaves which when abundantly infected, soon shrivel and fall, thus behaving differently from leaves infected with *Stagonospora meliloti*".

The species of Ascochyta and related fungi described on clover and lucerne

Reference*)	Host	Fungus	Country	Symptoms	Size of spores
(10)	*Medicago sativa*	*Ascochyta medicaginis* Fuck.	England	leaf spot	4-7μ by 1.8-2.9μ
(2)	*Medicago sativa*	*Phyllosticta medicaginis* Fuck.	Italy	leaf spot	5-7μ by 2.5-3μ
(9,10)	*Medicago sativa*	*Ascochyta imperfecta* Peck.	England	leaf and stem spot	10.6-15.4μ by 2.3-4.3μ
(3)	*Trifolium alpestre*	*Ascochyta trifolii alpestris* Dominik	Poland	leaf spot	9-15μ by 2-3μ
(6)	*Trifolium pratense*	*Ascochyta trifolii* Bond. et Trous. [*Stagonospora recedens* (Mass.) Jones].	England	leaf spot	15-22μ by 4.5-6μ
(6,8)	*Trifolium pratense* (8) *T. repens* (6)	*Stagonospora recedens* (Mass.) Jones	Italy U. S. A.	leaf and stem spot root rot	a) according to MASSALONGO(8): 16-24μ by 5-5,5μ b) according to JONES(6): 13.7-17.7μ
	Trifolium alexandrinum	*Ascochyta sp.*	Palestine	leaf and stem spot	15--20μ by 3-5μ

*) The numbers in this column correspond with those of the papers listed as references on p. 178

As apparent from the table, the fungus found in Palestine on Egyptian clover agrees in its symptoms and in the size of its spores with the fungus described by BONDARTZEFF as *Ascochyta trifolii*. However, the spores of this fungus were stated by JONES and WEIMER(6) to possess two septa and it was therefore referred by these authors to *Stagonospora recedens*. It cannot thus be identical with our fungus, the spores of which contain only two cells separated by a single septum. Unless further examinations reveal the occasional presence of an additional septum, the name *Aschochyta trifolii* will have to be applied to the species occurring in this country on Egyptian clover.

Perithecia of *Sphaerulina* were frequently found on the lesions on clover leaves or stems, in close association with pycnidia of *Ascochyta*. The relation of these two forms to one another is not yet clear.

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Dedicated to the memory of Aaron Aaronsohn on the 25th anniversary of his death.

ON THE DISEASE RESISTANCE OF WILD EMMER

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(With 1 Text-figure)

The discoverer of wild emmer (*Triticum dicoccoides* Koern.) in Palestine, AARON AARONSOHN (1), believed this wheat to be endowed by nature with numerous desirable properties not possessed by the cultivated species and varieties of wheat, especially as regards resistance to drought and diseases, and this belief was at first shared by COOK (2).

However, this view was challenged by later evidence. REED (6) was the first to prove, by accurate experiments, that wild emmer is subject to infection by powdery mildew (*Erysiphe graminis* DC.). VAVILOV (15) proved by inoculation trials that emmer is likewise attacked by leaf rust (*Puccinia triticina* Eriks.). In Palestine, we began in 1924 to investigate the susceptibility of wild emmer to covered smut (*Tilletia tritici* (Bjerl.) Wint.) This work was carried out not only with a view to adding to our knowledge of the range of diseases by which wild emmer is liable to be attacked, but also with the practical purpose of determining whether or not this wheat could be used for breeding a smut resistant variety of cultivated wheat.

The trials were carried out in 1924/1925 at Ben Shemen and in 1927/28 at Tel Aviv, both localities being situated in the central coastal plain of Palestine. The results of the 1927/1928 trial are presented here for the first time. The results of the earlier trial have previously been published elsewhere (8) but are again included for the sake of completeness.

The author is indebted to Agr. N. NAPHTHOLSKY and to Dr. F. LITTAUER, of the Division of Plant Pathology, for their assistance in the execution of this work.

THE EXPERIMENTS

The spore material used for infection was derived from smutted ears of Jiljilie wheat (*Triticum durum melanops*) collected at Ben

Shemen. The covered smut spores were dusted over the grains of the wild emmer after removal of the glumes.

In the 1924 experiments we used one type of wild emmer the exact variety of which was unfortunately not determined. The 1927 experiments comprised 3 different varieties of wild emmer (cf. table I). All these were collected at Rosh Pinah, in the Upper Galilee. The seeds were sown 5 cm apart in drills 20 cm apart. In 1924, 100 grains were sown on 12th December and developed without irrigation. In 1927, only 12 grains of each variety were at our disposal, and half of this number served as control, so that inoculation was tested on only 6 grains. In this trial the seeds were sown on 27th December 1927 and the plants were irrigated on alternate days.

The results of both experiments are presented in table I.

Table I

Infection of wild emmer by covered smut (Tilletia tritici)

Species and variety	Year	Locality	No. of plants sown	No. of plants infected
Triticum dicoccoides Koern.	1924	Ben Shemen	77	55
Tr. dicoccoides Koern. var. *spontaneo-nigrum* Perc.	1927	Tel-Aviv	6	3
Tr. dicoccoides Koern. var. *Kotschyanum* Schulz	"	"	5	4
Tr. dicoccoides Koern. var. *fulvo-villosum* Perc.	"	"	6	6

As apparent from the table, all the varieties of wild emmer were found to be infected by covered smut (cf. text-fig. 1).

Text-fig. 1. Wild emmer (*Triticum dicoccoides* var. *Kotschyanum* Schulz).

On the right: two sound ears.

On the left: two ears infected by covered smut (*Tilletia tritici*).

WILD EMMER AND THE DISEASE RESISTANCE OF WHEAT VARIETIES

It has long been assumed that the tetraploid species of wheat (28 chromosomes), to which hard wheat (*Triticum durum*) belongs, are marked by their resistance against smut and other diseases (4, 5, 10, 11). Some writers (9, 13, 14) went even further and considered disease resistance to be directly related to the number of chromosomes, the tetraploid species with 28 chromosomes being held to be resistant, while the hexaploid species with 42 chromosomes, including *Tr. vulgare,* were susceptible. Corroboration of this view appeared to be forthcoming in the experiments carried out by VAVILOV (17) and ZHUKOVSKY (18). VAVILOV found that *Tr. persicum,* previously regarded as belonging to the *Tr. vulgare* group, resembled hard wheat in its resistance to diseases, and cytological study actually showed *Tr. persicum* to possess only 28 chromosomes and to be thus a member of the *Tr. durum* group. ZHUKOVSKY discovered a new tetraploid wheat, *Tr. Timopheevi,* resistant against mildew, rust and smut attack.

However, the researches of THOMPSON (13) and STEVENSON (12) showed that this distinction between the resistance of tetraploid and hexaploid species of wheat does not apply in all cases: many hexaploid forms were found to be resistant and, conversely, many tetraploid forms proved susceptible to disease. Indications to that effect had, in fact, been given by VAVILOV (15) as early as 1918, when he mentioned the presence of both resistant and susceptible types in the species *Tr. dicoccum* and *Tr. dicoccoides.* Nevertheless, the species of wheat possessing 28 chromosomes may be said to include a larger number of resistant forms, the entire subspecies *expansum* being, for instance, resistant (16).

As regards wild emmer, the species comprises, according to JACUBZINER (3), no less than 19 varieties. Our experiments have proved at least 3 of these varieties to be susceptible to smut but in view of the observations made by AARONSOHN (1), COOK (2) and VAVILOV (15) on the resistance of wild emmer to disease, we may well assume that some of the remaining varieties possess this property to a marked extent.

In addition to the inherent disease resistance of the various varieties of wild emmer, development of diseases on a given variety may largely be affected by the presence or absence of those strains of pathogens to which that variety may be particularly susceptible. In the case of covered smut of wheat, at any rate, we have proved the existence in Palestine of a special strain (7). The failure of wild emmer to contract infection by various diseases in VAVILOV's (15) experiments may therefore have been due to the absence of particularly virulent pathogenic strains.

The results of our experiments thus do not necessarily in-

dicate general susceptibility of wild emmer to diseases; only a limited number of varieties of this wheat have been proved susceptible to infection by what was probably a single strain of covered smut. Other varieties of wild emmer may yet prove more resistant to this strain or, alternatively, the varieties we tested as well as others may be resistant to other strains of pathogens.

SUMMARY

In two field experiments carried out in the central coastal plain of Palestine, seeds of at least 3 varieties of wild emmer (*Triticum dicoccoides*), after being dusted with dry spores of covered smut (*Tilletia tritici*) collected on Jiljilie wheat (*Triticum durum melanops*), produced a high proportion of smut infected plants.

These findings are discussed in relation to the general problem of disease resistance of tetraploid and hexaploid wheat varieties. In view of the fact that each of these types has been shown to comprise resistant as well as susceptible forms of wheat, and considering the well-known existence of local strains of pathogens, which differ greatly in their virulence, two possible explanations are offered for the divergence of our results from the observations of other writers: Either wild emmer possesses, in addition to those tested by us, a number of genuinely resistant varieties, or Palestinian strains of some of the pathogens are particularly virulent and their absence abroad explains the failure of wild emmer to contract infection in other countries.

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OVERHEAD APPLICATION OF FUNGICIDAL SPRAYS

(Preliminary Experiments)

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INTRODUCTION

In Palestine, approximately one half of the area under vegetables and large areas of forage crops, ornamentals, tree nurseries, etc. are being irrigated by overhead irrigation systems. Two types of systems predominate: (a) pipe lines, usually of 60 metres length, revolving slowly from side to side around their own axis, with nozzles placed 60-90 cm apart, irrigating a belt of 7.5 metres width on either side; (b) sprinklers rotating fast on upright pipes of varying height, and irrigating each a circular area of about 10 metres' diameter.

The idea of utilizing these irrigation systems for the application of sprays was always very attractive to growers, but was largely vetoed on theoretical grounds. Sprays applied in this way obviously cannot be as finely dispersed as sprays applied directly by high-power sprayers, and there are little chances of covering the underside of leaves by means of sprays applied only from above. The efficacy of spraying, it was argued, cannot therefore equal that of power spraying.

These arguments, however, were not based on actual experimental evidence. On the other hand, the problem of finding a more convenient way of spraying became more and more urgent, as our trials with fungicides proved that only frequent applications succeeded in checking some of the major crop diseases under local climatical conditions. We therefore noted with great interest, that in England —where war-time conditions brought about a large increase in the areas of vegetables grown on small plots, with no spray equipment available — application of copper fungicides by ordinary watering cans successfully checked late blight (*Phytophthora infestans*) of potatoes (2). These findings encouraged us to test the efficacy of spraying by means of overhead irrigation systems, as compared with the efficacy of sprays applied in the ordinary way, in a series of field trials the first results of which are briefly summarized here.

Small motor sprayers (2.5-3 horse power) are to be found in most of the collective settlements of this country, and these settlements togeher command the greatest part of the area under overhead irrigation. The procedure adopted in our trials was to connect the outlet of the motor sprayer directly with one end of the irrigation pipe, into which the spray fluid was thus pumped under pressure. One difficulty was immediately encountered in the first tests: the small pumps did not suffice to supply the fluid to a revolving pipe of 60 metres length (66 nozzles) or to a large number of sprinklers. However, the first aim of these trials was not to develop practical means of spraying large areas through overhead irrigation systems, but to examine the phytopathological problem, whether this method of spraying can check diseases effectively, and whether it will be worth our while to devote subsequent efforts to the perfection of a suitable technique.

We therefore decided to test the efficacy of this method of spraying on small plots, the size of which was limited by the capacity of the motor pump. In practice, we found that small motors commonly employed here can supply spray fluid in quantities and with pressure sufficient to cover the entire width of the plot, only to revolving pipes of about 15 metres' length (18 nozzles) or to 2-3 sprinklers. Revolving pipes of this length were used in 4 trials, while 2 sprinklers were used in one trial. The rate of spraying was not fixed beforehand, but spraying was continued until the foliage was wetted thoroughly.

THE TRIALS

Three trials were carried out on potatoes and two on cucumbers. In two of the potato trials, directed against *Alternaria* leaf blight (*Alternaria solani*), and in the cucumber trials, designed for the control of downy mildew (*Peronoplasmopara cubensis*), we used the locally produced proprietary copper spray Floridan, which contains approximately 8% metallic copper in ammoniacal solution. In the third potato trial, Cita Californian spray, a proprietary lime-sulphur spray of 30-32° Baumé was used for the control of powdery mildew (*Oidium sp.*). In each of these trials the efficacy of spraying through the overhead irrigation systems was tested in comparison with the ordinary method of spraying by motor pumps usual in this country, where the plants are sprayed by means of long rubber hoses inserted longitudinally between the rows.

The first trial on potatoes, was purely preliminary in character; the efficacy of spraying was therefore determined by field estimations only, and yields were not recorded. In all the remaining trials, estimations were supplemented by full yield data. But, owing to the difficulties arising in the technical execution of these treatments, full replication (4 plots) was possible only in the two potato trials;

in the two cucumber trials we had to content ourselves with one and two replicates, respectively.

A. Potatoes

Trial No. 1, Giv'ath Brenner (Central coastal plain, south of Tel-Aviv), summer 1943.

A plot sized 11 by 15 metres was sprayed with Floridan (2%) through the revolving pipe type of overhead irrigation; a plot of comparable size was sprayed with Floridan in the usual way and two similar plots served as controls. A total of 4 applications was made, the rate of spraying amounting to approximately 450-600 litres per dunam (=1000 sq. metres) on the former, and 180 litres per dunam on the latter plot.

The plots were mainly attacked by *Alternaria* blight. At the final estimation, one third of the leaves on the control plots had dried up while the remaining leaves were affected with blight lesions on at least one third of their surface. On the sprayed plots, on the other hand, the proportion of dried leaves did not exceed 10%; among the lower leaves some were affected up to one third of their surface, but the majority showed only few lesions of *Alternaria* blight, and most of the upper leaves were free from disease. The state of infection of the plot sprayed through the irrigation system exactly equalled that of the plot sprayed in the usual fashion.

Trial No. 2, Giv'ath Brenner (Central coastal plain, south of Tel-Aviv), autumn 1943.

All treatments were replicated 4 times. The arrangements were the same as in trial No. 1, and Floridan 2% was again used for spraying by overhead irrigation. A total of 4 applications was made, the rate of spraying averaging 450 litres per dunam on the plots sprayed through overhead irrigation, as against 210 litres on the remaining plots.

The plots were attacked by *Alternaria* blight only. The blight was estimated on a scale of categories and marks resembling that recently described for other crops (5) and ranging from "traces" (0.1 mark) to "severe defoliation" (8 marks). The effect of Floridan spraying, in either fashion, and of Bordeaux mixture 1%, applied in the usual way, on the incidence of blight and on the size and grade composition of the yield appears in Table I.

Table I. Effect of treatments on the incidence of Alternaria blight on potatoes and on size and grade composition of yield

(each figure represents the average of 4 plots)

Treatment	Incidence of Alternaria blight		% size of yield (control=100%)	% of tubers in grade A (total yield per treatment =100%)
	marks (maximum incidence= 8 marks)	% (control =100%)		
Control	7.0	100	100	79.7
Floridan 2%, overhead application	1.75	25	108	83.6
Floridan 2%, ordinary spraying	1.1	16	93	80.0
Bordeaux mixture 1%, ordinary spraying	1.0	14	96	82.4
significant difference (P=0.05):			26.9	8.79

The table shows that all treatments greatly reduced the incidence of Alternaria blight, but failed to affect yields appreciably. This was due to the lateness of the blight attack, but need not concern us here. As regards the subject of this paper, applications of Floridan by overhead irrigation are seen to have been very slightly less effective than ordinary spraying in checking the development of the fungus causing *Alternaria* blight, but the fungicidal efficacy of the spray applied in the novel way was nevertheless entirely satisfactory. We further note that the effect on the yield of the spray applied by overhead irrigation was slightly better than that of the sprays applied in the ordinary way, but the difference was not significant.

Trial No. 3, Kfar Gileadi (upper Galilee), autumn 1943.

The arrangement of this trial resembled that of trial No. 2, and there were 4 replicate plots (sized 15 by 4.5 metres) to each treatment. As the trial was designed for the control of powdery mildew the treatments tested comprised sulphur sprays and dust. A total of 6 applications was made; the rate of application was not recorded.

The plots were attacked by powdery mildew, the incidence of which was estimated by the scale of categories and marks used in all our trials against this disease (3). Table II indicates the effect of treatments on the incidence of disease and on the size and grade composition of yield.

Table II. Effect of treatments on incidence of powdery mildew on potatoes and on size and grade composition of yields

(each figure represents the average of 4 plots)

Treatment	Incidence of powdery mildew		% size of yield (control =100%)	% of tubers in grade A (total yield per treatment =100%)
	marks (maximum incidence =8 marks)	% (control =100%)		
Control	0.88	100	100	74.2
Cita Californian Spray 1.5%, overhead application	0.05	6	117	80.0
Cita Californian Spray 1.5%, ordinary spraying	0.05	6	111	78.5
significant difference (P=0.05):			19.0	17.5

Here again, the spray applied through the overhead irrigation system was as effective as ordinary spraying, and again we note the slight, but not significant, increase in yield on the plots sprayed by the former, as compared with those sprayed by the latter method.

B. Cucumbers

In the trials described above, the efficacy of spraying through overhead irrigation was tested with diseases attacking the leaves from either surface, such as *Alternaria* blight, or infecting first of all the haulms, such as powdery mildew, and the trials were carried out on potatoes which are a bushy and at least semi-erect crop. In these cases the spray applied purely from above had a reasonable chance of covering a large proportion of the leaf and stalk surfaces exposed to infection. We further tested the efficacy of the novel way of spraying in a less favourable case, against the downy mildew disease of cucumbers (*Peronoplamopara cubensis*). As cucumbers are not staked in Palestine, the crop is entirely prostrate. The downy mildew disease is known to infect the leaves through their lower surface (1), and particular care in applying sprays to that surface has always been advocated in the control of that disease. It was therefore of interest to determine, to what extent sprays applied through the overhead irrigation system would succeed in checking this mildew.

Trial No. 4, Ain Hashofet (Eastern ranges of Mount Carmel), autumn 1943.

Two replicate plots, each measuring 7.5 by 15 metres, were sprayed with Floridan 1% through an overhead irrigation system of the revolving type. The

remaining treatments, Floridan 1% and Perenox ¼%, sprayed in the ordinary way, were each applied to 4 replicate plots. The sprays were applied 8 times, the rate of application by overhead irrigation averaging 450 litres per dunam, while the remaining plots were sprayed at the rate of 150-200 litres per dunam.

The plots were severely attacked by downy mildew. The effect of the above treatments on the incidence of disease was estimated by the method recently described elsewhere (5). Table III presents the results of this estimation and the yield data.

Table III. Effect of treatments on incidence of downy mildew of cucumbers and on the yield

Treatment	Incidence of downy mildew: marks (maximum incidence =8 marks)	Incidence of downy mildew: % (control =100%)	% size of yield (control =100%)	
Control	5.12	100	100	(average of 4 plots)
Floridan 1%, overhead application	2.96	58	429	(average of 2 plots)
Floridan 1%, ordinary spraying	3.16	62	482	(average of 4 plots)
Perenox ¼%, ordinary spraying	3.3	64	403	(average of 4 plots)

All treatments gave only partial control of the disease, but the spray applied through the overhead irrigation system was no less effective than the sprays applied in the ordinary way, and yield increases were approximately equal on all the treatments plots.

Trial No. 5, Mizpe Jam, (Central coastal plain, north of Tel-Aviv), autumn 1943.

One plot, measuring approximately 15 by 10 metres, was sprayed with Floridan 1% by means of two sprinklers. For comparison, Floridan 1% and Perenox ¼% were each applied to 2 replicate plots. The sprays were applied 5 times, the rate of spraying approximating 200 litres per dunam on the plots sprayed in the usual fashion, but 500-600 litres per dunam on the plot sprayed through the sprinklers.

The attack of downy mildew was so destructive, that the control yield was negligible. The effect of treatments on the incidence of disease (estimated as in trial No. 4) and on the size of yield appear in table IV.

Table IV. Effect of treatments on incidence of downy mildew of cucumbers and on the yield

Treatment	Incidence of downy mildew		% size of yield (control =100%)	
	marks (maximum incidence =8 marks)	% (control =100%)		
Control	6.77	100	100	(average of 2 plots)
Floridan 1¼%, overhead application	4.16	61	1021	(1 plot)
Floridan 1¼%, ordinary spraying	4.73	70	718	(average of 2 plots)
Perenox ¼%, ordinary spraying	4.27	63	1015	(average of 2 plots)

Here, as in trial No. 4, downy mildew control was only partial. Overhead application, this time through sprinklers which distribute the spray in a finer form than revolving pipes, was again as effective as ordinary spraying and appeared to at least equal the latter in its effect on the yield.

Both the above cucumber trials show that the application of sprays through the overhead irrigation system may succeed in checking the downy mildew disease to the same extent as ordinary spray applications. This result may appear surprising, if we recall that the organism causing cucumber downy mildew penetrates the leaves through their undersides (1). The same problem has frequently been referred to in literature in connection with the downy mildew (*Plasmopara viticola*) of grape vine. Control of this disease has been practiced for a long time, especially in France, by means of sprays applied only from above and covering only the upper leaf surface, although the disease is known to penetrate almost solely through the undersurface of the leaves. In the opinion of some writers (4) the at least partial success of sprays applied from above may be explained by the fact that the wind-borne spores settle at first mostly on the upper side of the leaves; there the sprays may kill them even before they manage to reach the lower leaf surface whence they penetrate the leaf. This explanation might be thought to apply in the case of cucumber downy mildew as well, and may furnish the reason for the successful control of the latter disease by sprays applied through the overhead pipe system.

CONCLUSIONS

The trials briefly outlined above show that, from a phytopathological point of view, the application of fungicidal sprays through overhead irrigation systems merits further study as a means of controlling some destructive vegetable diseases. The small motor pumps at present in use in most settlements do not suffice to supply the spray solution to entire pipe lines of the usual length (50-60 metres), but stronger pumps should be able to do so. In the trials reported here overhead application of sprays proved effective against powdery mildew (*Oidium sp.*) and Alternaria blight (*Alternaria solani*) of potatoes and against cucumber downy mildew (*Peronoplasmopara cubensis*). Further trials will have to show the efficacy of this mode of application against other crop diseases.

The practical advantages of the overhead application of sprays are manifold, and may be summarized as follows:

(1) The labour required amounts to less than one third of that required for ordinary spraying of an equal area.

(2) Injury to the crops, ordinarily caused by the wheels of the motor sprayer or the dragging of hoses, may be avoided entirely.

(3) Sprays may be applied at any time, even when the ground is wet. This may be most important where spray applications must be timed to be carried out immediately after the rain or after irrigation.

These advantages are likely to outweigh by far the chief draw-back of the overhead application of sprays, viz. the large amount of spray materials used.

SUMMARY.

Three trials on potatoes and two trials on cucumbers have been carried out to test the efficacy of fungicidal sprays applied through overhead irrigation pipes, as compared with the usual methods of motor spraying practiced in Palestine.

Overhead application of sprays satisfactorily controlled *Alternaria* blight (*A. solani*) and powdery mildew (*Oidium sp.*) of potatoes and downy mildew (*Peronoplasmopara cubensis*) of cucumbers and in all cases gave yields at least equal to those obtained by the usual spraying methods.

The rate of application of sprays applied through the overhead irrigation system was 2-3 times as high as the ordinary rate of spraying. However, this draw-back is considered to be outweighed by the many advantages of the novel mode of spraying, which are discussed briefly.

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Dedicated to Dr. S. Lehmann, Principal of Ben Shemen Youth Village, on his 50th birthday

STUDIES ON MUSHROOMS AND OTHER FUNGI OF THE FORESTS OF PALESTINE

III. An edible forest truffle, its taxonomy and geography

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(With Plate V and 3 Text-figures)

I. INTRODUCTION

The countries of the southern and eastern Mediterranean are known to harbour a wealth of truffles, and most species of the genus *Terfezia* have been found there. In Palestine, however, we have hitherto been unable, in the course of twenty two years of mycological work, to discover a mushroom that could be included in this group, the only hypogaeous fungi encountered being *Gastromycetes*.

The absence of truffles in Palestine appeared to us to be due to ecological causes. On the one hand, the northerly types of truffles of the genera *Tuber*, *Elaphomyces*, *Cheiromyces*, etc. could not be expected to occur here any more than in other countries of the eastern and southern Mediterranean, because they require a very moist climate which obtains in these countries only in isolated localities. On the other hand, the settled parts of Palestine, where we have searched for mushrooms, belong to the macchia region and

would not seem to suit the southerly types of truffles or terfas, as they have been called by DUGGER (4) to distinguish them from the former type. The terfas mushrooms prefer a dry and hot habitat, such as the steppes of the mauritano-turanic region (12), which has also been termed the irano-turanic region (5). DUGGER, who explored the habitat of the species of *Terfezia* in North Africa, found them to occur in typical *Artemisia herba alba* steppes. These mushrooms were therefore not likely to occur in those parts of Palestine which possess a typically Mediterranean climate, while they might be found in the steppic parts of the country, in the south, or in certain parts of Transjordan.

In view of all these considerations we were greatly surprised when an edible truffle was found two years ago in the *Pinus halepensis* forest at Ben Shemen, near Tel Aviv, and the question arose, whether this mushroom belonged to the northerly group of genuine truffles or to the southerly group of terfas.

The mushroom was discovered by Mrs. Batsheva MARGOVSKY, of Ben Shemen, an ardent collector of mushrooms growing in the forests of that vicinity, to whom we are also indebted for description of the various ways in which the truffle may be prepared for consumption. The author is obliged to Dr. A. BONDI, of the Division of Chemistry of the Agricultural Research Station, Rehovot, for the analytical examination of the nutrient value of the truffle. We further with to thank Dr. Z. AVIZOHAR and Dr. M. CHORIN, of the Division of Plant Pathology, for their assistance in the examination of the fungus and in the preparation of the plates and drawings. J. WAHL, of Ben Shemen Youth Village, kindly assisted us in our observations.

II. DESCRIPTION AND HABITAT

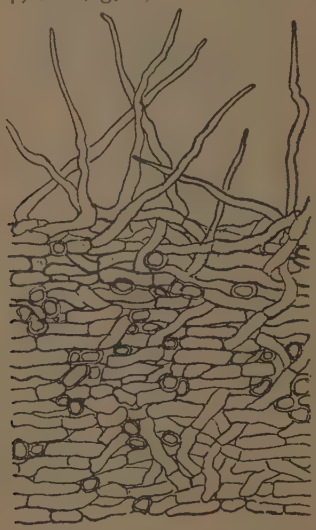
The ascocarp is irregularly globose, without special point of attachment, humpy or slightly folded, measuring usually 3-4 cm, but sometimes up to 10 cm in diameter; smaller fructifications, measuring only 1-2 cm in diameter appear at the end of the season. All types shrivel after some time. The surface of the ascocarp is smooth, nonlustrous, and of light clay colour (pl. V, fig. 1).

In transverse section the gleba appears sometimes dark brown, but usually of coconut-like whitish colour which darkens after some exposure to the air. The gleba is traversed by white veins of unequal width (40-400 μ). The veins emerge from the similarly textured rind, ramify irregularly either forming meshes or terminating in blind ends (pl. V, fig. 2).

The gleba is situated between the veins, and the asci are embedded in its tissue. The asci are formed singly, each in a small cavity the walls of which are 1.5-3.6μ thick (pl. V, fig. 3). Each ascus exactly fits its cavity, which collapses if the ascus is removed;

there does not, therefore, appear to be a special membrane lining these cavities (pl. V, fig. 5). The ascus contains 1-4 spores, mostly 1-2 spores. Examining 250 asci we found 52 per cent. with 1 spore, 32 per cent. with 2, 13 per cent. with 3, and only 3 per cent. with 4 spores (pl. V, figs. 3, 5).

The tissue of the rind is seen in section to be composed of hyaline hyphae arranged more or less tangentially to the surface of the ascocarp. Among these hyphae there appear glittering rings, resembling plectenchymatic cells, which represent transverse sections of hyphae running in the same plane in all directions. On the outer side of the rind some of the hyphae composing the latter extrude and form a layer of fibrils attenuated at their ends; these fibrils are 50-70μ long and 1.5-7μ wide (pl. V, fig. 3; text-fig. 1). The veins, as mentioned above, run more or less radially from the rind towards the centre of the ascocarp and are textured like the rind. Within the veins hyphae run in general longitudinally and are grouped loosely in bundles. This is apparent from the group of glittering rings apparent in sections, which are bundles of hyphae cut transversely (pl. V, fig. 4; text-fig. 1).



Text-fig. 1. Digrammatical section through the rind of an ascocarp of *Delastriopsis oligosperma*, showing fibrils and rind tissue composed of tangential hyphae. The rings apparent between these hyphae are transversely cut ends of hyphae running in all directions (x 540).

The hyphae are 1-10μ broad and septate, the septa being 3-30μ apart. The hyphae are as a rule continuous, but sometimes irregular in shape (text-fig. 1).

The asci are subglobose or ovoid, with a small, curved protuberance at one end. The overall length of asci amounts to 60-110 μ and their width to 45-80 μ. The protuberance is 5-17 μ long and 10-14 μ broad. The spores are at first hyaline or yellowish, but turn brown when mature; they are ornamented with an alveolate structure composed of a periscope and spines borne at the corners of its polygonal cells. The diameter of the spores (without spines) amounts to 18-50 μ, 90 per cent. of the spores examined measuring 30-45 μ. The perispore is about 1.5 μ thick. The spines 3-3.75 μ long and their base is 4.5-7.5 μ wide. The spines are placed 4.5-8 μ apart. The diameter of the polygonal cells of the alveolate structure amounts to 1.8-6 μ (pl. V, fig. 5; text fig. 2).

Text-fig. 2. Diagram of ascus of *Delastriopsis oligosperma* containing two spores with spines and perispore (x 220).

The observation that the gleba appears sometimes light and sometimes dark in colour may find its explanation in the change of colour taking place in the spores as they mature. A whitish gleba seems to contain mostly young spores, while a gleba containing mature spores will appear dark. In any case, no differences of either size or shape could be detected in the examination of 250 spores from either kind of gleba.

The truffle was found 3-5 cm deep in heavy, calcareous soil under *Pinus halepensis* trees. The fruit bodies first appear in March and continue to be found until late April. They usually occur in groups of 6-10 per tree, but the groups are sometimes larger and up to 120 specimens have in one instance been collected under a single tree. The only outward sign of the presence of these truffles is the appearance of cracks and a slight elevation of the soil.

III. TAXONOMIC POSITION

According to the above description the truffle belongs to the family *Terfeziaceae*. This family is characterized by the presence of only a single type of veins, which are limited to the inner portion of the fruiting body and do not reach its surface, and contrasts with the *Tuberaceae* which possess two types of veins traversing the cortex to reach the outer portion of the fruiting body. Our truffle, by the irregular arrangement of the asci in masses separated by veins and by its alveolate spores, approaches the genus *Terfezia* itself. TULASNE (14), who first found this mushroom in France, referred it to this genus and created for it a new species, *Terfezia oligosperma*. ED. FISCHER (6) and BATAILLE (1) also mention the fungus under this name.

However, this determination does not appear entirely satisfactory, because the truffle in question differs from the remaining species of the genus *Terfezia* by one important characteristic: the asci contain only 1 to 4 spores, and not 8 spores as in the other species of the genus. In this respect our mushroom approaches another genus of the *Terfeziaceae*, the genus *Delastria*, in which the asci contain 1-4 spores. The first to notice this distinction was MATTIROLO (11). He accordingly removed the fungus from the genus *Terfezia* and, to suit its description, created a new genus which he called *Delastriopsis* to indicate its resemblance to the genus *Delastria*. BATAILLE (1) did not agree with MATTIROLO's decision and left the fungus in the genus *Terfezia*.

In our view, MATTIROLO was justified in separating the fungus denoted as *T. oligosperma* from the genus *Terfezia*. The difference in the number of spores per ascus is fundamental and distinctive, and in this respect our fungus approaches the genus *Delastria*. The question therefore arises, why did MATTIROLO prefer not to include *T. oligosperma* in this genus, but to create a new genus for this mushroom. In fact, CLEMENTS and SHEAR (3) went further than MATTIROLO, cancelled his genus *Delastriopsis*, and considered the resemblance in the number of spores per ascus to be sufficient for the inclusion of the fungus in the genus *Delastria**).

But this view does not appear acceptable in view of the marked morphological differences between the genera emphasized by TULASNE (14) and MATTIROLO (10). The rind of *Delastria* is stated by these authors to possess a silky, cottony, fibrillose integument

*) CLEMENTS *and* SHEAR (3), incidentally, were not justfiied in including *Delastria*, and thus also *Delastriopsis* which they consider synonymous with *Delastria*, in the group of *Hyalosporae*. As described by MATTIROLO (10) and apparent also from ED. FISCHER'S (6) drawings, the spores of this genus are not hyaline but dark yellow. This also applies to MATTIROLO's genus *Delastriopsis* (cf. the preceding chapter).

which is absent in *Delastriopsis* as well as in *Terfezia*. Speaking of *Delastria*, TULASNE (14) states "il suffit d'un leger frottement pour faire disparaitre le voile bryssoide qui recouvre cette jolie Tuberacée et pour la rendre meconnaissable." The absence of such an integument apparently also decided TULASNE to refer *T. oligosperma* to the genus *Terfezia*. Our mushroom certainly has no cottony integument such as the one described above. As apparent from fig. 3 in table V, there is, in fact, a hairy covering upon the rind, but this does not resemble a special integument and cannot be rubbed off, as is stated by TULASNE to be the case in the genus *Delastria*. Our fungus definitely approaches the latter genus, and it is remarkable that FISCHER (6), who examined *Delastria* and published a drawing of a tranverse section of its peridium, did not indicate a special integument in this genus, and merely shows the rind to be covered by short fibrils similar to those in our figure. On the other hand, another transverse section of the whole fruit-body of *Delastria* drawn in the same paper, shows the presence of a point of attachment. This is absent in *Delastriopsis* and would represent an important morphological difference between the two genera, but this feature has been mentioned neither by TULASNE (14) nor MATTIROLO (10) nor by FISCHER (6) himself in his description of *Delastria*. Only a comparative study of these mushrooms will be able to show whether or not the two genera are identical. For the time being, we propose to follow MATTIROLO who denoted the mushroom in question as *Delastriopsis oligosperma*. If future examinations will prove the fungus identical with *Delastria* this truffle will have to be called *Delastria oligosperma* (Tul.) Reicht. nov. comb.

The genus *Delastriopsis* may thus be characterized by the small number (1-4) of spores per ascus and resembles *Terfezia* in outward appearance. According to this definition, one further species, *Terfezia Gennadii*, should be transferred from *Terfezia* to *Delastriopsis*. This species was created by CHATIN (2), who found the mushroom in Greece, and was confirmed by BATAILLE (1) to belong to the genus *Terfezia*. It resembles *Delastriopsis oligosperma* by the small number of spores per ascus. BATAILLE recognized this resemblance by placing the two mushrooms in a separate group within the genus *Terfezia*, but in the light of the above definitions it should obviously be transferred to *Delastriopsis*, to be re-named *Delastriopsis Gennadii* (Chat.) Reicht. nov. comb. This second species of the genus *Delastriopsis*, according to BATAILLE, differs from *D. oligosperma* by having elongated asci, whereas the asci of *D. oligosperma* have above been described as ovoid or globose.

IV. AREA AND ECOLOGY

Delastriopsis oligosperma has so far been found in southern France and Sardinia (1). The habitat of this mushroom has been

described only from France, where it occurred in an oak forest (9). The truffle has not been discovered in Spain or north-western Africa, where so many other types of truffles have been found to occur (text-fig. 3).

Text-fig. 3. Distribution of *Delastriopsis oligosperma* (black dots) and *D. Gennadii* (black triangle).

The second species of the genus, *Delastriopsis Gennadii*, has hitherto only been discovered in the Peloponnese peninsula of Greece, where it developed on light soil covered by grass vegetation, and its occurrence was limited to the early spring months (text-fig. 3).

According to the distribution of its two species, the genus *Delastriopsis* may thus be designed as a n o r t h - e a s t e r n M e d i t e r r a n e a n c o m p o n e n t .

As regards the ecological requirements of the genus, the habitat of *D. oligosperma* in Palestine and southern Europe indicates preference for conditions of high moisture and the absence of low winter temperatures. As mentioned above, the truffle occurs in this country in a pine forest on heavy soil, at Ben Shemen, where annual rainfall approximates 500-600 mm. Our searches for this mushroom in the pine forests of the Lower Galilee where partly owing to the nature of the soil, conditions are somewhat drier, proved fruitless. In the localities in which the truffle occurs in southern Europe, in France and Sardinia, annual rainfall is also considerable and approaches 700 mm. Moreover, the related species, *D. Gennadii*, is found on the Peloponnese where rainfall approaches the same figure and which is surrounded on all sides by the sea.

The most conclusive evidence of the hygrophilous nature of these truffles lies, however, in the fact that they develop only in winter or early spring, i. e. during the rainy period. If we further take into account that the truffles are hypogacous and develop only

underground, their formation in winter clearly indicates their affinity for moisture.

Concerning the temperature requirements of the truffles, their absence from northern countries may be taken to show their preference for moderate temperatures, or at least for climates in which winter temperatures are not too low.

The above considerations lead us to define the genus *Delastriopsis* with its two species as an oceanic-warm type.

V. ORIGIN AND MIGRATION.

Two of the facts emerging from the previous sections have to be taken into consideration in tracing the origin of the genus *Delastriopsis*: the species of this genus (a) are of an oceanic-warm type, and (b) do not occur in North Africa, where the truffle flora has been explored thoroughly and many other species were found to be present. It is therefore reasonable to assume that *Delastriopsis* has its origin in southern Europe. This assumption is supported by the following considerations: (1) Both the species at present included in the genus have been found in southern Europe. (2) As pointed out above, further study may show the genus *Delastria* to be identical with *Delastriopsis*, and the two genera are in any case closely related to one another. The occurrence of *Delastria* in Southern Europe therefore tends to confirm our view of the origin of *Delastriopsis* in that region. *Delastriopsis* may thus be defined, according to the locality of its origin, as a south-European element.

If we consider the geographical origin of *Delastriopsis* to be thus established, certain implications concerning the period at which the genus formed are at once apparent. During the middle miocene and early quarternary epoch southern Europe was linked with North Africa by two land bridges, one passing through Italy and Sicily, the other through Corsica and Sardinia. These bridges served for the passage of thousands of plants, which are to-day found in Europe as well as Africa. The fact that *Delastriopsis* truffles do not occur in North Africa renders it probable that the genus formed only after the period in which these bridges existed, i. e. in the course of the quarternary epoch. *Delastriopsis* or its fore-runners may be thought to have at one time grown in the north, where numerous members of the *Tuberineae* are still found to-day, under oceanic-warm conditions. With the beginning of the ice age, the genus *Delastriopsis* may have migrated to the south until it reached southern France, where a more or less warm and oceanic climate persisted throughout the ice age. Although temperatures fell and the amount of rainfall increased greatly, the early forms of our truffles may well have maintained themselves over this period. Difficult conditions, we may imagine, were encountered by these mushrooms in the interpluvial period, when temperatures rose and atmospheric humidity

decreased until a desert-like climate prevailed (7). It appears plausible to suppose that these dry conditions induced the truffles to assume their hypogaeous mode of development, to escape drought and the scorching rays of the sun. The fore-runners of *Delastriopsis*, undetected primitive forms of the genus, could well have grown above ground until the hypogaeous form developed in response to the xeric conditions of the inter-pluvial period. A corresponding evolution appears, in the light of recent discovery, to have taken place in the genus *Terfezia*. All the species of this genus known until a short while ago were hypogaeous and occurred in xeric climates. But in 1934 an epigaeous species of *Terfezia* was discovered by HEIM (8) in Madagascar. It is our hypothesis that all the primitive forms of *Terfezia* were epigaeous and occurred in tropical climates, and only their descendants growing in the steppes assumed an hypogaeous form of growth, and that a similar development took place in the genus *Delastriopsis*.

We conclude from all these considerations that *Delastriopsis* probably originated in one of the interpluvial periods, and designate the genus as an interpluvial element.

One of the two species of *Delastriopsis*, *D. oligosperma*, migrated from the regions of its origin in southern Europe in a south-easterly direction and reached Palestine, most likely by way of Anatolia and Syria, where it may yet be found to occur. *D. oligosperma* must accordingly be defined as a north-east Mediterranean migrant. This migration may be assumed to have taken place during the quarternary epoch in the pluvial, but not in the dry interpluvial, periods. It follows that, from the point of view of the period of its migration, *D. oligosperma* is to be considered as a pluvial migrant.

VI. DISCUSSION

The above outline of the geographical distribution of the genus *Delastriopsis* in the present and the distant past, and the definition of its ecological requirements, affords a clear-cut reply to the question posed in the introduction to this paper, viz. whether our mushroom is a genuine truffle of the northerly type or belongs to the southerly type occurring in steppes and termed "terfas" by DUGGAR (4). By virtue of its origin and ecological amplitude the genus *Delastriopsis*, with both its species, must be included in the group of genuine truffles. From an ecological point of view the genus thus somewhat approaches the *Tuberaceae*.

The occurrence of a truffle belonging to this northerly ecological type has not previously been recorded in south-easthern Mediterranean countries, where only terfas, i. e. species of *Terfezia*, have so far been known.

VII. CULINARY VALUE

The mushroom described above is of agreeable flavour and odour, especially when it is young and its flesh white. The odour deteriorates when the mushroom grows stale and the flesh turns dark. The truffle may be prepared for consumption in many different ways. It may be roasted and may serve as spice for soups and other dishes, or it may be ground, fried in oil and dished up in form of cutlets, or may even be cooked like meat. In whatever way it is served, this truffle retains its distinctive flavour. In the months of February and March the mushroom represents quite an important addition to the diet of the villagers living near the forests in which it occurs.

To determine the nutrient value of the truffle, its water, protein, fat, and carbohydrate contents were analyzed by Dr. BONDI, who also calculated the calories it contains. The results of these analyses are presented in the following table, side by side with corresponding data for other foodstuffs, as given by STEPP (13).

The nutrient value of Delastriopsis oligosperma as compared with some other foodstuffs and mushrooms.

	Percentage content of				Calories per kg.
	water	protein	fat	carbohydrates	
Cattle meat	79	20	10	0	1730
wheat flour	15.5	11.8	1.5	71.0	3500
potato	75	2.1	0.1	21.0	960
cauliflower	91	2.5	0	4.0	270
beans	89	3.0	0	6.0	370
Psalliota	90	5.0	0.2	3.0	340
Boletus	87	5.0	0.4	5.0	430
Delastriopsis oligosperma	88	4.9	0.3	4.2	400

As apparent from the table, the nutrient value of *Delastriopsis oligosperma* is fair, and approaches that of *Psalliota* and *Boletus* and of beans and cauliflower.

VIII. SUMMARY

A detailed description is given of a truffle collected in Palestine in a forest of *Pinus halepensis*.

A review of the taxonomic position of this truffle shows it to belong to the genus *Delastriopsis* and to the species *D. oligosperma* (Tul.) Matt. Another truffle found in Greece and hitherto known as *Terfezia Gennadii* Tul. was likewise referred to the genus *Delastriopsis* and was re-named *D. Gennadii* (Tul.) Reicht. nov. comb.

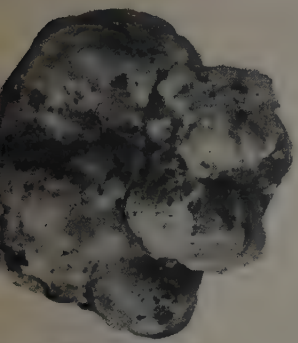


Fig. 1.

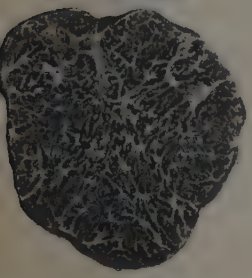


Fig. 2.

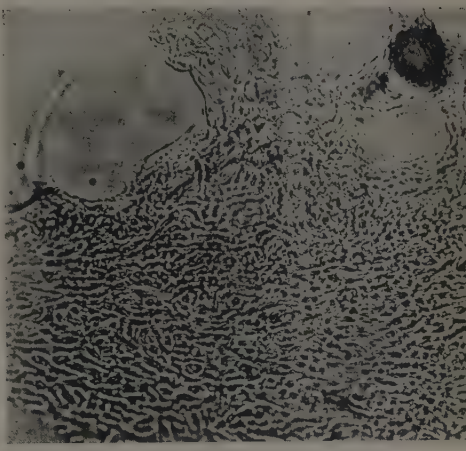


Fig. 4.

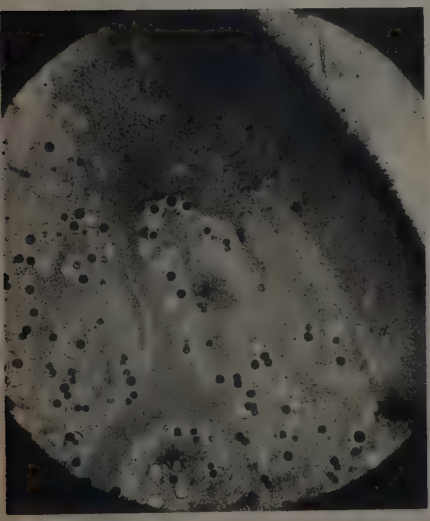


Fig. 3.

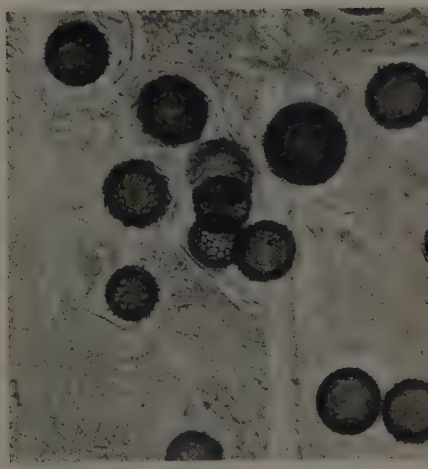


Fig. 5.

From the distribution and ecology of these two species the genus *Delastriopsis* is concluded to be a north-eastern Mediterranean component and an oceanic-warm type.

Tracing the origin and migration of the truffle, the genus *Delastriopsis* with both its species is designated as a south-European and interpluvial element. The species *D. oligosperma* is defined as a north-east Mediterranean and pluvial migrant.

D. oligosperma is concluded, according to its ecology, origin, and distribution, to belong to the northerly group of genuine truffles, and not to the group of terfas mushrooms occuring in southern countries.

The culinary value of *D. oligosperma* is discussed. Analyses have shown its nutrient value to equal that of *Psalliota* and *Boletus* mushrooms and of beans and cauliflower.

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EXPLANATION OF PLATE V

Delastriopsis oligosperma

Fig. 1. Ascocarp (nat. size).

Fig. 2. Section through ascocarp. White veins are seen to ramify in the dark tissue of the gleba (nat. size).

Fig. 3. Section through outer tissues of ascocarp. The following structures are visible, proceeding from the outside towards the centre: fibrils, dark rind continued in similarly textured veins, and whitish gleba and spores (x 50).

Fig. 4. Section through the rind, showing fibrils and rind tissue composed of tangential hyphae. The glittering rings apparent between these hyphae are transversely cut ends of hyphae running in all directions (x 440).

Fig. 5. Section through gleba, showing cavities with asci and spiny spores (x 350).

NOTES

THE OCCURRENCE OF *USTILAGO NIGRA* ON BARLEY IN PALESTINE

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Observations made by the author over a number of years in various parts of Palestine have shown that loose smut of barley is more prevalent on Arab than on Jewish farms. Jewish farmers always treat the seed with dry seed dressings, while this practice is not as yet established among Arab farmers, and the impression was formed that the seed dressings succeed in partially controlling the loose must disease. However, this impression was irreconcilable with the fact that the organism hitherto assumed to be solely responsible for loose smut of barley in Palestine, *Ustilago nuda* (Jens.) Kell. et Sw., is known not to be amenable to control by dry seed dressings.

Considerable interest therefore attached to TAPKE's discovery (Phytopathology 22:869-870,1932) of a second organism, *U. nigra* Tapke, which causes loose smut of barley by seedling infection and is controllable by seed dressings. This discovery led us to undertake a survey of the loose smuts of barley occurring on some of the Jewish farms in various parts of Palestine, including the southern, cenral, and northern parts of the coastal plain, the Valley of Esdraelon, the Beisan Valley, and the Upper Galilee. A total of 31 samples of barley ears affected with the disease were collected between 20th March and 4th May, 1944. The samples were kept in newpapers at room temperature (up to 30°C) until the 18th - 26th of June, when the chlamydospores were germinated.

The method employed to germinate the spores resembled that described by TAPKE (Phytopath. 31:281-286, 1941; ibid. 33:194-209, 1943). A suspension of spores in water was poured on to potato dextrose agar in Petri dishes. The water was poured off after a few minutes, and the dishes were kept at a temperature of 23-24°C. The mode of spore germination was examined after 14-18 hours.

The germinating spores could be divided into two distinct types. In one type the percentage of germination was low, the rate of germination slow, and the spores germinated into a branch-

ed mycelium. Spores of the second type germinated abundantly and rapidly and bore masses of sporidia. The first type corresponds to TAPKE's description of *U. nuda*, the second type to that of *U. nigra*. The cultures of *U. nuda* were mostly contaminated with bacteria of an unidentified species.

Of the 31 samples examined, 11 cases of loose smut were found to be caused by *U. nigra* and the remaining 20 by *U. nuda*. The two species of loose smut were sometimes found in a single locality on the same variety of barley. Either type of loose smut therefore seems to be widespread in Palestine. In view of the fact that *U. nigra* has been stated to be controllable by surface disinfectants, the application of seed dressings to barley seeds assumes increased importance.

OBSERVATIONS ON *SCLEROTINIA SCLEROTIORUM* IN PALESTINE

By ZAPHRIRA ELAZARI-VOLCANI*)

Occurence. Rots of roots, stalks, and fruits are commonly caused in Palestine by *Sclerotinia sclerotiorum* (Lib.) Mass. Among those most important, mention may be made of fruit and stalk rot of marrows and eggplants, and fruit rots of bananas and oranges (cf. REICHERT, Internat. Bulf. Pl. Protect. 13:75-81, 226-240, 277-293).

The disease is permanently extending the range of its hosts and represents a serious menace to economic crops in this country.

Inoculation. In a small scale inoculation test to prove the virulence of the fungus on tomatoes, five seedlings raised in pots in sterilized soil were inoculated with mycelium interspersed with sclerotia. The inoculum originated from diseased tomatoes and was grown on sterilized barley kernels. After 16 days, one of the inoculated plants contracted the disease, and sclerotia were found to be present in the pith of its wilted stalk. All check plants remained healthy.

Apothecia. Apothecia were very rarely found to form from sclerotia in the field, but the cold winter of 1941/42 afforded us a good opportunity of making some observations in this respect. Apothecia formed on citrus fruits affected by *S. sclerotiorum*, after they had been transferred to the laboratory, and were also found (at Mikve

*) At present serving with the Palestine contingent of the Auxiliary Territorial Service somewhere in the Middle East.

Israel, near Tel Aviv) on the soil of a plot of vegetable marrows and of a tomato seed-bed, in which the plants were affected by the disease. Apothecia further formed in the above mentioned tomato experiment on the soil of the pots inoculated with the fungus.

Measurements of the apothecia gave the following figures: the diameter of the disc ranged from 3 to 15 mm, the length of the stalk from 5—35 mm. The spores measured 9—12μ in length and 4.5—6.5μ in width. Apothecia usally formed in clusters of up to 6 individual cups.

Temperature. In an experiment to determine temperature relationships of the fungus, sclerotia were sown in Petri dishes on glucose agar and the rate of lateral extension of the mycelium was recorded for 4 days. Two sources of mycelium, from tomatoes and cucumbers, were used and two cultures of either were raised at each of the following temperatures: 1, 4, 10, 12, 14, 20, 25, 30, 35, and 37°C.

No growth was made at 1,35 or 37°C, while growth was slight at 4 and 30°C, better at 10 and 12°C, and best at 14,20 and 25°C. These results on the whole agree with those reported from abroad; but there are some minor differences, as indeed there are between the findings of various investigators in other countries. A comparative study of *S. sclerotiorum* with material derived from various geographical sources appears necessary.

DIAPHANOSCOPE FOR THE EXAMINATION OF CULTURES IN PETRI DISHES *)

By G. MINZ,

Division of Plant Pathology, J. A. P. Agr. Res. Sta., Rehovot

In the routine examination of Petri dish cultures these have often to be raised for inspection by transmitted light. This is particularly necessary with black mycelia, and when fruiting bodies have to be examined and selected for mounting, or transference. The inconvenience of such handling is obvious and when cultures are on a liquid medium the procedure is impracticable. A device facilitating the examination of Petri dishes by transmitted light is described here.

A round 10-Watt electric lamp, 40 mm. in diameter, is arranged in a tin or wooden box measuring 14 to 14.5 cm. square and

*) Received for publication in February 1942.

4 to 4.5 cm. in height. The light is transmitted to the Petri dish through a round hole in the top of the box, measuring 8 to 10 cm. in diameter and covered by a square of frosted glass inserted through

Text - fig. 1. Diaphanoscope for the examination of cultures in Petri dishes.

a slit in the side of the box. A switch is affixed to the box or to the wire near it, so that the lamp may readily be switched off while the Petri dish is left in place without being heated unduly. A few small holes on the sides of the box serve for ventilation. (Text-fig. 1).

OBITUARY

Sir EDWIN JOHN BUTLER

The death of Sir Edwin John BUTLER, on 4th April 1943, has robbed his innumerable friends all over the world of a truly great man. BUTLER was the first to make phytopathological and mycological science transcend its narrow limitation to northern countries. He systematically explored the causes of plant diseases in tropical regions and gave this branch of science its global foundation. Effective and fruitful penetration into the depth of any science is possible only after the white spots on its map have been filled in, and after coordination of all the known facts. BUTLER succeeded in filling in many of the "white spots" of phytopathology in his time, and in coordinating the work of pathologists the world over. Without his efforts the rapid advance of plant disease research witnessed in his life time would have been unthinkable. His work in crop diseases in tropical and subtropical countries is counted among the corner stones of mycological science.

When, in 1921, I began mycological research in Palestine and asked Professor TRINCHIERI, then head of the mycological section of the International Institute of Agriculture, at Rome, for literature on plant diseases in warm countries, I was answered that there was but one such book: BUTLER'S "Fungi and Diseases in Plants". Even now, after 22 years, this book remains fundamental for the knowledge of plant diseases outside the countries of the north.

BUTLER was far more than a mere organizer of mycological research in the British Empire, huge as this task in itself may appear. His personality and the institutions he created (Imperial Mycological Bureau, Review of Applied Mycology) were implicitly trusted everywhere and were consulted whereever scientific or practical emergencies arose. BUTLER was at home with the mycological problems of India, Africa, and America, no less than with those of Great Britain. He was the cosmopolitan pathologist par excellence. At the botanical congress held at Amsterdam in 1935 it was BUTLER who knew almost every mycologist in person, or at least by correspondence. Indeed, he was among those present like an uncrowned king, or rather he was crowned, in a sense, by almost all the highest honours which such a congress may bestow.

BUTLER was always kindly disposed towards our mycological work in Palestine. From the beginning of this work until he left the Imperial Mycological Bureau he stood by our side and gave us the benefit of his personal interest and of his enormous fund of knowledge. Even at a later period, when he left the Bureau to become the Secretary of the Council for Agricultural Research, he helped us on numerous occasions. His support induced the Empire Marketing Board to award us, in 1928, a 3-year grant for the study of citrus fruit rots, and if this research is still continued now, under the auspices of the Government of Palestine, we certainly, on that account as well, owe a debt of gratitude, to BUTLER, who was the mycological adviser of the Colonial Office.

We consulted BUTLER on all intricate problems arising in this country, where mycological research was new, and disease control problems were pressing and required an approach different from that in northern countries. His advice always enlightened us and bore the stamp of his wide knowledge and limitless experience.

Humanity and wisdom, seasoned by humour, emanated from him as he collected around him, for a daily cup of tea at 11 o'clock in the morning, most the members of his staff and the visiting mycologists. Whoever was fortunate enough, as I was, to attend these informal gatherings, will never forget them.

In 1931, we were privileged to welcome BUTLER to Palestine, on his return from the Sudan to England. Here he exhibited all the variety of his cultural and intellectual interests. He visited most parts of the country and enquired closely into our ways of agricultural settlement, so different from the ways of colonisation in other parts of the Empire which he knew so well. He took special interest in the new sociological forms of our collective and cooperative settlements and in the cultural advances made in this country. After visits to the Hebrew University and the National Library, at Jerusalem, he twice attended performances of the Habimah theatre and, as he wrote to us later from England, was especially impressed by their Hebrew rendering of Shakespeare's 'Twelfth Night". When he met Colonel KISH, then chairman of the Zionist Executive, BUTLER was pleasantly surprised to find in him not only a man with an intimate knowledge of India, but also a relative of Professor Marcus HARTOG, who was his first teacher of botany and had awakened his early interests in mycology.

The phytopathological and mycological sciences at large have lost one of their great founders, and we, in our modest way, have lost a devoted friend. His memory will ever keep alive in science, and his friends and admirers will never fail to remember him.

I. R.

YOHEBED BEN-MEIR — GLUECK

The Research Station at Rehovot, and in particular the Division of Plant Pathology, has suffered a heavy loss by the untimely death of YOHEBED BEN-MEIR—GLUECK. A young life of exceptional promise was curtailed, after only 34 years, by a cruel road accident that occurred on the 13th August 1942.

YOHEBED BEN-MEIR—GLUECK, born in Russia, was educated in Palestine and France. After completion of her studies she worked as secondary school teacher for natural history. But attracted by purely scientific work she joined, in 1937, the staff of the Division of Plant Pathology, where her work was marked by outstanding skill and devotion. Participating in the scheme of citrus wastage investigations sponsored by the Palestine Government, she was engaged mainly in the survey of fruit rots carried out in hundreds of orange groves and in the study of the biology of fungi causing stem-end rots. She made a special study of the fungus flora present in the air of orange groves and of packing sheds and, by demonstrating the presence of spores of the causal agents of various orange rots, proved the danger of fruit contracting infection while in the packing sheds. Another subject of her researches was the determination whether the principal varieties of *Citrus* are susceptible to infection by inoculum of the stem-end rot fungus *Diplodia natalensis* deriving from other species of the genus and from other hosts

outside that genus (cf. the paper published on pp. 162-165 of the present issue of this Journal). All the main types of citrus fruits were found to be infected by *Diplodia* from any other type, and stem-end rot of Shamouti oranges was induced by inoculum from numerous other hosts; the presence of the fungus on almost any host thus constitutes a threat to citrus fruits.

Yoheved Ben-Meir—Glueck, in addition to her scientific work, had many and varied interests, especially in the fields of sociology and pedagogy, and approached these and other subjects with an original and independent mind. Her warm humanity was the cause of her popularity among her colleagues and of her happiness with her husband and her two infant children. A most tragic fate has torn her from our midst: we shall never forget her.

מכשיר חדש לבדיקת תרבויות

מאת ג. מינץ

(עם תמונה בדף 208)

ניתן תאור של מכשיר חדש לבדיקה נוחה של תרבויות בצלחות פטרי ע"י הארה מלמטה.

סיר אדוין ג'ון בטלר ז"ל

למדע הפיתופתולוגי והמיקולוגי אבד אחד מגדולי יוצריו. סיר אדוין ג'ון בטלר מי שהיה ראש המכון המיקולוגי של האימפריה הבריטית נפטר באפריל 1943. הוא הוציא את המדע הזה מגבולותיו הצרים של ארצות הצפון בהכניסו למעגל החקירה את מחלות הארצות הטרופיות. הוא ישב בהודו כ־20 שנה וב־1920 חזמינוהו ללונדון כדי שיעמד בראש המכון האימפריאלי למיקולוגיה. שם הוא יסד את העתון הסוקר את כל הספרות העולמית של המיקולוגיה ומיסדו נעשה למרכז עולמי של המדע הזה. לעצתו פנו הפיתופתולוגים מכל קצות החבל.

סיר א. בטלר בקר בא"י בשנת 1931 וסייר את הישובים החקלאיים החדשים, בקר באוניברסיטה ובספריה הלאומית והתענין בחיים התרבותיים בארץ. למחלקה לפתולוגיה הוא הגיש את עזרתו עד יומו האחרון. הוא השיג למחלקה את תמיכת ועדת השיווק של האימפריה לשם מחקר רקבונות פירות הדר. זכרו לא יסוף מקרב המדע עד עולם.

ר.

יוכבד בן־מאיר־גליק ז"ל

תוארה פרשת חייה של יוכבד בן מאיר־גליק ז"ל שעבדה במחלקה לפתולוגיה משנת 1937 עד ה־13 באבגוסט 1942, היום שבו נקפד פתיל חייה באסון דרכים כששבה הביתה מעבודתה. מסירותה לעבודה וכשרונה המדעי התבלטו בזמן עבודתה הקצרה.

היא היתה עסוקה במחקר רקבונות ת"ז וגורמיהם הטפיליים. היא עשתה תצפיות בהרכב הפטריות המצויות באוירה בפרדסים ובבתי אריזה וגם עבדה בקביעת כוחה הפתוגני של *Diplodia natalensis* על עצי הדר שונים.

זכרה לא ישכח לעולם ע"י חבריה.

שטח התפוצה והאקולוגיה של שני מיני *Delastriopsis* הנ"ל מראים כי הסוג הזה הוא קומפוננט צפון־מזרח ים־תיכוני וטיפוס ימי־חם. עקבנו אחרי העבר של הכמהה ובאנו לידי המסקנה כי אפשר לסמן את הסוג *Delastriopsis* על שני מיניו מבחינת מקום התהוותו כאלמנט דרום־אירופאי ומבחינת זמן התהוותו כאלמנט דילוביאלי. מבחינת דרך נידודיו אפשר לסמן את המין *D. oligosperma* כמגרנט צפון־מזרח ים־תיכוני, ומבחינת זמן הנידודים האלו כמיגרנט פלוביאלי.

בהתאם למקורו, תפוצתו ודרישותיו האקולוגיות שייך המין *D. oligosperma* לקבוצת הכמהים האמיתיים הנפוצה בארצות הצפוניות ולא לקבוצת ה„טרפס" המצויה בארצות הדרום.

מציאות שני מינו פחמון פורח בשעורה בארץ ישראל

מאת ג. מינץ

בבדיקת 31 דוגמאות של פחמון פורח על שעורה, אשר נאספו באביב 1944 בכל אזורי הארץ, נתקבלו התוצאות הבאות: ב־20 מקרים נקבעה כגורם המחלה הפטריה *Ustilago nuda* שנחשבה עד עכשיו למעורר היחידי של מחלה זו בארץ; אך ב־11 מקרים התברר כי המחלה נגרמה ע"י פטרית פחמון שניה בשם *U. nigra* שעל מציאותה הודיעו עד כה רק מארצות הברית. צורת המחלה, הנגרמת ע"י שני המעוררים משתווה והם נבדלים רק באופן נביטת נבגיהם וצורת הדבקתם. חשוב לציין כי — בניגוד ל־*U. nuda* — אפשר לפי תוצאות מחקר אמריקאי להדביר את ה־*U. nigra* ע"י חטוא חיצוני של הזרעים.

(מאמר זה נדפס במלואו בעברית ב„השדה" כרך כ"ד, חוב' י"ב, תש"ד).

תצפיות במחלת הקשיוניה הגדולה בא"י

(SCLEROTINIA SCLEROTIORUM)

מאת צ פרירה אלזרי־וולקני*)

נמסרו תוצאות הסתכלויות ונסיונות אחדים בביולוגיה של הפטריה *Sclerotinia sclerotiorum* המעוררת רקבון בהרבה ירקות ופירות שונים. נסיון הדבקה בשתיל עגבניות חולל את המחלה בצמחים.

נמצאו גופי פרי (אפותיקיות) שיצאו מן הקשיונות של הפטריה הנ"ל. הם הופיעו באדמה של שדה קשואים ושל מנבטת עגבניות שבהן נוגעו הצמחים ע"י המחלה, וגם באדמת עציצים אשר הדביקו בתפטיר ובקשיונות הפטריה. נקבע הגדל המדויק של האפותיקיות ושל חלקיהן השונים.

הפטריה לא גדלה כלל בתרבות בטמפרטורה של 1, 35 ו־37 מעלות צלזיוס, וגדלה בצורה חלשה ב־4 ו־30 מעלות. הגדול הטוב ביותר נתקבל ב־14, 20 ו־25 מעלות.

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*) חברת המחלקה לפתולוגיה של התחנה לחקר החקלאות, רחובות, שהתגייסה לצבא העזר לנשים.

בשני נסיונות שדה עם שלשה זנים שונים של חטת הבר הוכחנו כי זנים אלו רגישים להדברת הפחמון הסגור (*Tilletia tritici*).

תוצאות אלו עומדות בניגוד לממצאים של חוקרים שונים בחוץ לארץ, אשר מצאו את חיטת הבר לחסונה בפני מחלות שונות. את הסתירה הזו אפשר להסביר כדלקמן:

א) נוסף לשלשת הזנים שעמדו בנסיונותינו יש לחטת הבר עוד 16 זנים אחרים; יתכן שבין אלו נמצאים גם זונים חסונים והם אשר נבדקו בנסיונות בחו"ל.

ב) כידוע קיימים בארץ ישראל גזעים מיוחדים ותקיפים ביותר של מעוררי מחלות מסויימות. יכול להיות שבכחם של זני חטת הבר לעמד בפני התקפת גזעי המחלות הנמצאות בחו"ל, אך הם נדבקים ע"י הגזעים המקומיים.

השימוש במיתקני ההמטרה לרסוס נגד מחלות צמחים

מאת י. פלטיאל. מלר

ריסוס בתמיסות פונגיצידיות, ע"י חיבור מרסס מוטורי עם ראש צנור ההמטרה ושאיפת התמיסה מן הדוד ישר אל הצנור, נתן תוצאות שוות לריסוס מוטורי רגיל בהדברת החלפת (*Alternaria solani*) והקמחון (*Oidium sp.*) של ת"אד ובהדברת הכשותית (*Peronoplasmopara cubensis*) במלפפונים.

כמות התמיסה שרוססה בשיטת ריסוס זו עלתה פי 2—3 על הכמות הרגילה (כ־500 לעומת 200 ליטר לדונם), אולם יתרונות השיטה, אם נצליח לפתר את הבעיות הטכניות הקשורות בה, הם גדולים מאד: קימוץ שני שליש העבודה, מניעת נזק לצמיחה וריסוס טוב גם כאשר האדמה רטובה.

(מאמר זה נדפס במלואו בעברית ב„השדה", כרך כ"ד, חוב' י'—י"א, תש"ד).

מוקדש לד"ר ש. להמן,
מנהל כפר הנער בן־שמן,
לרגל יובלו החמישים

מחקרים בפטריות היערות של א"י

III. כמהת מאכל בא"י

מאת י. דיקרט

(עם לוח מס' V ו־3 תמונות בדפים 195, 196, 199)

בשנת 1942 מצאה הגב' ב. מרגובסקי ביערות האורן בבן שמן כמהת מאכל אשר לא נמצאה עד עכשיו בארץ. לכמהה זו יש טעם מצויין ואפשר להכין אותה למאכל באופנים שונים. ערכה המזוני שוה לזה של ארניות ושל כרובית ושעועית.

ניתן תאור מפורט של הכמהה, ושמה המדויק נקבע כ־*Delastriopsis oligosperma*. גמצא שכמהה אחרת, הנמצאה ביון וידועה בשם *Terfezia Gennadii*, שייכת לאותו הסוג *Delastriopsis* ושמה הנכון יהיה מעתה *D. Gennadii*.

נסיונות בהדברת הפחמון הסגור של השעורה ע"י חטוא זרעים

מאת י. ריכרט, ג. מינץ ו. פלטי

בששה נסיונות שדה להדברת הפחמון הסגור של השעורה (*Ustilago hordei*) נתקבלו תוצאות מצויינות ע"י החטוא היבש בתכשיר הכספית אגרוזן ג', בכמות של 2 גרם לק"ג זרעים. חטוא רטוב קצר בתמיסת כספית (כספן) לא הצליח להדביר את המחלה במידה הרצויה.

בנוגע לחטוא ע"י אבקת גפרית אישרו תוצאות הנסיונות את הדעה המקובלת בחו"ל, כי אפשר להדביר את הפחמון הסגור של השעורה ע"י חטוא יבש בגפרית. אולם נראה, כי השפעת הגפרית מוגבלת בלבד; בתנאי הדבקה קלה מספיקה פעולת הגפרית; אך בתנאי הדבקה יותר קשים, כגון במקרה שמקור הזרעים בשדה נגוע קשה בפחמון, אין בכוחה של הגפרית להדביר את המחלה לגמרי. משום כך יש להשתמש בגפרית כאמצעי חטוא נגד הפחמון הסגור של השעורה אך ורק כאשר ישנו בטחון כי הזרעים אינם נגועים במידה ניכרת בנבגי הפטריה.

(מאמר זה נדפס במלואו בעברית ב„השדה", כרך כ"ד, חוב' י"ב, תש"ד).

כתמי עלים וגבעולים בתלתן אלכסנדרוני

מאת מתילדה חורין

(עם תמונה בדף 175)

מחלת הכתמים בעלי התלתן ובגבעולו התפשטה בשנים האחרונות בכל הארץ. הכתמים מופיעים לראשונה בצדם התחתון של העלים ונראים אז כנקודות חומות כהות ושקועות במקצת, מוקפות בדרך כלל ע"י חצר צהובה. אחרי זמן קצר נראים הכתמים גם בצדם העליון של העלים: מרכז הכתמים אז הופך לצבע חום בהיר, יבש ומופיעים עליו לפעמים עגולים קונצנטריים, בעוד ששפת הכתמים נשארת חום כהה והחצר מסביבם נעלמת. לבסוף מתאחדים כתמים רבים וגורמים להתייבשות העלים. סימני המחלה בגבעולים דומים לאלו בעלים, אך הכתמים מאורכים יותר בצורתם.

לפי גודל נבגיה ומספר תאיהם שייכת הפטריה הגורמת לכתמים אלו אל הסוג *Ascochyta*. השוואת הפטריה עם תאורם של גורמי מחלות דומות בתלתן ובאספת בארצות אחרות נותנת יסוד לחשב כי היא שייכת למין *A. trifolii*.

מוקדש לזכרו של אהרן אהרנסון למלאת 25 שנה למותו.

על חסינות חטת הבר כלפי מחלות

מאת י. ריכרט

(עם תמונה בדף 180)

ניתנה סקירה על הדעות ששלטו לפנים, אחרי שאהרון אהרנסון גלה את חטת הבר בארץ ישראל, על מידת עמידותה של חטה זו כלפי מחלות.

יאגסים. היות ותנאים אלו אינם מצויים בזמן לבלוב הזנים האירופאיים, על כן אין הם נתקפים ע"י הפטריה הזאת.

רק תנאי האקלים הם המונעים, איפוא, את התהוות המחלה בזנים האירופאיים ולא חסינותם המיוחדת.

הדבקת פירות הדר ע"י *DIPLODIA* מאכסנאים שונים

מאת ג. מינץ | ויוכבד בן־מאיר

נסיונות הדבקה הוכיחו כי רקבון העוקץ מתפתח בפירות תפוחי־זהב שמוטי אם מדביקים אותם בנבגי *Diplodia* מהענפים של מיני הדר שונים או מ־21 אכסנאים מחוץ לסוג *Citrus*. רק נבגי הפטריה מתמר, *Mentha piperita* ו־*Pyrus syriaca* לא גרמו להתפתחות הרקבון.

רקבון העוקץ של פירות אשכולית ולימון נגרם ע"י הדבקה בנבגי דיפלודיה מעץ הקקיון ומשטה (*Acacia farnesiana*), אך הדבקות בדיפולדיה שבודדה מתמר היו שליליות. דיפלודיה מענפי כל מיני הדר החשובים בארץ־ישראל גורמת לרקבון העוקץ של פירות תפוחי־זהב, אשכוליות ולימונים.

לאור התוצאות הנ"ל אפשר להניח כי דיפלודיה הבאה כמעט מכל מקור שהוא מהווה סכנה לפירות הדר.

חקירה אנאטומית בעקצי תפוחי זהב ביחס לרקבון העוקץ

מאת מינה גדל

(עם תמונה בדף 167)

בחקירות אנאטומיות בעקצי תפוחי זהב לא נמצא אף פעם תפטיר פטריות ברקמות הפנימיות של העוקץ, לעומת זה נמצא בחיק עלי הגביע תפטיר בתוך הרקמה הקרועה והנקרוטית הנוצרת במקום האבקנים ועלי הכותרת, ולפעמים גם בחלקים חיצוניים אחרים של העוקץ.

מציאות התפטיר במקומות אלו מלווה תמיד בהתהוות שכבת שעם המפרידה בין הרקמה הנקרוטית והתפטיר אשר בתוכה לבין הרקמה הבריאה של העוקץ. הוכחנו בנסיונות כי שכבת השעם נוצרת גם סביב פצעים מכניים (דקירות מחט) וגם מסביב למקומות החדירה של פטריות.

הסתכלויות נוספות, בקשר עם חקירות תרבותיות מקבילות, נותנות יסוד להניח כי התפטיר הנמצא בשכבות החיצוניות של העקצים שייך לפטריות גורמי רקבון העוקץ (*Alternaria*, *Diplodia*) או *Collectotrichum*). מציאות תפטיר כזה מהוה, איפוא, סכנה להדבקה לטנטית ע"י הרקבונות האלו בשעת האסום.

הטמפרטורה האופטימלית להתפתחות מעורר המחלה בתרבות היא ב־25—30 מעלות צלזיוס והמקסימום ב־40 מעלות. בנסיונות הדבקה בטמפרטורות של 25 — 40 מעלות לא הצליחה הפטריה להדביק פקעות ת'אד, אפילו אחרי פציעה. לעומת זה הצליחו ההדבקות שנעשו לאחר חימום הפקעות ב־55 מעלות במשך שעתים. מהתוצאות הנ"ל נובע כי הרקבון בת'אד עלול לתקף את הפקעות רק אחרי שניזוקו ע"י חום גבה. טמפרטורות קרקע גבוהות בגידול ת'אד אביביים אפשר למנע ע"י השימוש בזנים מוקדמים, ע"י זריעה מוקדמת ואסיף מוקדם, ע"י זריעה בקרקע מתאים ולא קל ביותר, ע"י השקאות תכופות יותר וע"י המשך ההשקאה עד זמן קצר לפני האסיף. את הנזק באיסום אפשר להפחית ע"י טמפרטורות קרות המונעות התפתחות מהירה של המחלה.

נסיונות בהדברת הקמחון בתפוחי אדמה

מאת י. פלטיש. מלוד

ארבעה נסיונות הראו כי ריסוס בתכשירים גפרית־סידיים (מרק קליפורני סיטה, סולפינט) בתרכוזת של 1.5% מטיב להדביר את הקמחון (*Oidium sp.*) בת'אד. גם האבקה לריסוס ספרסול, אשר אפשר להוסיפה לתמיסת פרנוקס, נתנה תוצאות טובות, וגם איבוק בגפרית עזה מסוג אקסטרא פיין היה יעיל. לעומת זה היתה השפעת הריסוסים הנחושתיים (מרק בורדו, פרנוקס וכו') קטנה. הריסוס במרק גפרית־סידי גרם לצריבות קלות בעלים.

כפי שנראה מתוצאות הנסיונות אפשר להדביר את הקמחון בת'אד ע"י ריסוס או איבוק בתכשירים גפריתיים בהפסקות של שבועיים, החל מהופעת הסימנים הראשונים ועד 3 — 4 שבועות לפני האסיף. מחלת הקמחון מפחיתה את היבול רק כאשר היא מופיעה בצמחים בשני החדשים הראשונים אחרי הזריעה. המחלה עלולה אז להמעיט את כמות היבול ב־20% או יותר ולהשפיע לרע גם על הרכב היבול לפי סוגי השוק.

התפוצה של מחלת הגרב בתפוחים ואגסים בארץ־ישראל

מאת י. פרלברגר

(עם 2 דיאגרמות בדפים 159 ו־160)

מחלת הגרב בתפוחי עץ ואגסים, הנגרמת ע"י הפטריות *Fusicladium dendriticum* ו־*F. pirinum*, מצויה בארץ בכל אזוריה. היא תוקפת את העלים ואת הפירות.

עד עתה נמצאה המחלה רק על זנים מקומיים של תפוחים ואגסים. הזנים המקומיים מלבלבים ופורחים מוקדם בעונה, בתקופה שבה שוררת טמפרטורה נמוכה והגשמים בה שכיחים. הזנים האירופאיים, אשר הוכנסו ארצה בשנים האחרונות, מלבלבים שבועות אחדים יותר מאוחר, כשחלפה כבר תקופת הגשמים. בתקופה זו שכיחות גם טמפרטורות גבוהות ולחות יחסית נמוכה. נגבי ה־*Fusicladium* מסוגלים, לפי KEITT ו־JONES, לנבט רק כשהם בתוך מים וכשהטמפרטורה נמוכה ורק אז עלולים הם לתקף עלים ופירות של תפוחים

נסיונות שדה בהדברת מחלות עלים של עגבניות

מאת י. ריכרט, י. פלטיאל, מינץ,
בהשתתפות ב. כפולך וש. סטולר

ב־7 נסיונות להדברת מחלות הקמחונית (*Leveillula* (*Oidiopsis*) *taurica*) ועבש העלים (*Cladosporium fulvum*) של עגבניות סתויות וחרפיות בבית זרע, דגניה א', ניר דוד ומקוה ישראל נתקבלו התוצאות הבאות:

ריסוסים בתכשירים גפרית־סידיים (מרק קליפורני סיטה, סולפינט) בתרכוזת של 1.5% היו יעילים מאד בהדברת הקמחונית, בשעה שריסוסים נחושתיים השפיעו עליה רק במידה קטנה. בהדברת עבש העלים קבלנו תוצאות טובות בתכשירים הגפריתיים הנ"ל, בסולפוסייד בן 0.5%, בתכשירים הנחושתיים מרק בורדו ביתי בן 1% ופרנוקס בן 0.5% או 0.33% עם או בלי תוספת שמן לבן בן 1%, ובשירלן אג בן 0.5%.

בנסיון אחד בניר דוד הפחיתו הריסוסים הנחושתיים במידה ניכרת את מספר הפירות הקפואים. הריסוס בפרנוקס עם שמן לבן הקטין את נזק הכפור בעלים, בשעה שכל יתר הטיפולים לא השפיעו על מידת נזק זה.

הדברת הקמחונית ע"י סולפינט התבטאה בהגדלת היבול של 50% (מ־2830 ל־4250 ק"ג לדונם). בנסיונות נגד עבש העלים הוגדל היבול במידה רבה ע"י ריסוסים בתכשירים הגפרית־סידיים וגם ע"י ריסוס בפרנוקס עם שמן; אולם הריסוס בפרנוקס בלבד (0.33%) או במרק בורדו בן1% לא הגדיל את היבול בהרבה. יש לחשב שריסוסים אחרונים אלו הוזקו במקצת לעלי העגבניות.

הטיפולים שהצליחו להדביר את הקמחונית ואת עבש העלים האריכו בדרך כלל את עונת ההנבה בהרבה. פעולה מיוחדת במובן זה יש כנראה לשמן לבן.

מהנסיונות בעמק הירדן הסקנו את המסקנה כי להדברת הקמחונית מספיקים ריסוסים בהפסקות של שבוע ימים, החל מהופעת המחלה. לעומת זה הראו הנסיונות במקוה ישראל כי עבש העלים אינו ניתן להדברה אלא ע"י ריסוסים תכופים, פעם ב־4—5 ימים. בתנאי עמק הירדן עולה הגדלת ההכנסות, עקב ההדברת הקמחונית, בהרבה על הוצאות הריסוסים. בתנאי מקוה ישראל תלויה כדאיות הריסוסים ברמת היבול ובמחירי השוק.

(מאמר זה נדפס במלואו בעברית ב„השדה", כרך כ"ג, חוברות ב'—ח').

קשיון הבטטה (*SCLEROTIUM BATATICOLA*) בתפוחי אדמה בארץ־ישראל

מאת פ. ליטאור

(עם 2 תמונות בדפים 142 ו־143)

קשיון הבטטה גורם בארץ ישראל לכמישת גבעולי ת'אד, לרקבון שרשיהם ולשני מיני רקבונות בפקעות, והם רקבון יבש של הטבור ורקבון הפחם. מחלות אלו, ביחוד רקבונות הפקעות, מופיעות בעיקר בחלקות מזריעת אביב מאוחרת, בסוף הגדול ובשעת האסיף. רקבון הפקעות הנגרם ע"י קשיון הבטטה בשעת האסום מגיע עד כדי 50%. כל זני ת'אד נפגעים ע"י המחלה.

בסיסיים. מידת ה־pH האופטימלית היא 6 — 6.6. הפטריה מפרישה כמות ניכרת של חומצה אוקסלית, וגם פקטינזה. הצליחו לגדלה על מספר קרקעי מזון נוזלים טבעיים וסינטטיים.

הקשיונות נבטו יותר מהר באד מאשר בחושך. קשיונות שנאספו מהקרקע או מצמחים נגועים לא איבדו את כשר נביטתם אפילו אחרי שנה. הקשיונות נמצאו חסונים במידה רבה להשפעת חום יבש ונבטו אפילו אחרי חימום ב־80 מעלות במשך 30 דקות וב־110 מעלות במשך 5 דקות.

הדבקה מלאכותית של חסה, שעועית ותפוח אדמה בשדה ובמעבדה חוללה את סימני המחלה הרגילים. גם בפירות תפוחי זהב, קלמנטינות, תפוחי עץ, אגסים ובננות הצליחה ההדבקה בלי פציעה. מנסיונות אחדים בפונגיצידים שונים נראה כי הקשיוניה הקטנה רגישה יותר להשפעת תרכובות כספית מאשר לגפרת הנחושת או לתכשיר הגפרית „סולבר".

נסיונות שדה בהדברת מחלות הכשותית והקמחון במלפפונים

I. על היעילות של חמרי נחושת שונים

מאת י. ריכרט, י. פלטי וג. מינץ, בהשתתפות ב. כפולר

(עם תמונה בדף 109)

בדקנו בנסיונות שדה את פעילותם של חמרי נחושת שונים בהדברת הכשותית (Peronoplasmopara cubensis) והקמחון (Erysiphe cichoracearum) וגם את השפעתם על צמחי המלפפונים עצמם. נבדקו בעיקר מרק בורדו בן 1%, פרנוקס (מבוסס על תחמוצת של נחשת) בן ½3%, קופרוגרין אבק (אבקה נחושת־סידנית בת 8% נחושת) ותמיסת פלורדו. בסינו גם את ההשפעה של תוספת שמן לבן קל בינוני בן 1% לתמיסת הפרנוקס.

הערכנו את נגיעות החלקות בכשותית וקמחון ע"י קביעת דרגות נגיעות מסוימות לפי שטח העלה הנגוע.

בנסיונות שלא הופיעה בהם מחלת הכשותית הפחית השימוש במרק בורדו את היבול בזמן שריסוס בתמיסת פלורדו או איבוק בקופרוגרין אבק לא הפחית אותו. בנסיונות שנעשו בתנאים נוחים להתפתחות הכשותית מצאנו שאין להלחם במחלה זו ע"י טיפולים בהפסקות של 10 ימים, כמו שנהגו עד עכשיו, אבל הצלחנו לעצר אותה ע"י הקטנת ההפסקות עד ל־4 ימים. ריסוסים תכופים במרק בורדו עצרו את הכשותית אבל לא הגדילו את היבול. בזמן שריסוסים בתכשירים „בלתי־נמסים" (פרנוקס וקופרוגרין מרוכז) ואיבוק באבקת נחושת־סיד (קופרוגרין אבק) עצרו את הכשותית וגם הגדילו את היבול באופן מובהק. לתכשירי הנחושת המועילים נגד הכשותית יש גם המעלה שהם מאריכים באופן ניכר את תקופת האסיף. ריסוסים תכופים בפרנוקס מדבירים ג"כ את הקמחון.

הוספת שמן לבן לתמיסת פרנוקס הגדילה את היבול רק בתנאים שהצמחים סבלו מיובש, אולם האריכה תמיד את תקופת האסיף יותר מאשר הריסוסים הנחושתיים בלי שמן.

עתון לבוטניקה

כרך ד׳ חוב׳ ב׳ — סדרת רחבות — אלול תש״ד

חקירה בחידקים המייצרים חומצה בוטירית שבודדו מקנבס ומרקמות צמחים דומות

מאת חיים ויצמן ואסתר הלינגר

(עם לוחות מס׳ ו ו־חו).

נעשה מחקר בחידקים אנאירוביים המייצרים חומצה בוטירית שבודדו מקנבס, יוטה ופשתה מא״י, הודו ומנילה. ניתנה סקירה על הספרות הנוגעת בבעיה זו. ניתנו תאורים מורפולוגיים ותרבותיים מפורטים של 10 חידקים שבודדו. תסיסת תירס ע״י החידקים האלו חוללה בעיקר חומצות וולטיליות, אולם רק אחד מהם הראה פעולה דיאסטטית מהירה על פסולת אמילן. נחקר גם כוח ההתססה של החידקים האלו בתוך גלוקוזה ונעשו הערכות של מוצרי ההתססה. המוצרים הסופיים העיקריים הם אציטון, כהל בוטילי, איזופרופילי ואטילי, וחומצות וולטיליות. נוצרו גם חומצת חלב וחומצה פורמית בכמויות קטנות. התרבות שנתנה כמויות גבוהות של אציטון וכהל בוטילי מפסולת עמילן יצרה גם קרבינול אציטילי מטילי. ניתנו טבלאות השוואה בין כשר החידקים להתסיס מולסות ופסולת חלב רזה.

כל החידקים שייכים לסוג *Clostridium*, ששה מהם שייכות לקבוצת *Cl. acetobutylicum* (Weizmann), אחד מזדהה עם *Cl. butyricum* Prazmowski והאחרים שייכים ל־*Cl. pectinovorum* Stoermer. בתוך המין *Cl. butyricum* McCoy נמצאו שני גזעים חדשים, האחד v. *elongatum* והשני v. *acetobutylicum*. חוץ מזה נמצא גזע חדש במין *Cl. pectinovorum* ונקרא v. *parvum*.

הקשיוניה הקטנה (*SCLEROTINIA MINOR*) על חסה ועל שעועית

מאת דבורה סרנה

(עם לוח מס׳ IV ותמונה בדף 88)

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